THE
AMERICAN NATURALIST

VOL. LXXXIV March–April, 1950 No. 815

OBSERVATIONS ON TWO CASES OF INTERSPECIFIC HYBRIDIZATION WITH *DROSOPHILA ATHABASCA*

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INTRODUCTION

Drosophila athabasca Sturtevant and Dobzhansky (1936) is one of the relatively small, dark-bodied flies assigned to the *obscura* group (*affinis* subgroup) of the subgenus *Sophophora* (Sturtevant, 1942). This species has a rather wide distribution in North America. In the eastern United States it has been collected in New England and the Great Lakes region and in the mountains of North Carolina and Tennessee. In the west, collections have been made at various places along the Pacific coast (Oregon to Alaska) and in the Rocky Mountain region from New Mexico to central Alaska. It is quite possible that further collections will show the species to have a more or less continuous distribution across the northern Great Plains. (For collection points of this and the other *affinis* subgroup species used in this study, see Sturtevant and Dobzhansky, 1936; Miller, 1939; Sturtevant, 1940; Patterson, Wagner, and Wharton, 1943; and Novitski, 1946.)

In the laboratory, *Drosophila athabasca* may be made to hybridize with three other species of the *affinis* subgroup. It was reported by Sturtevant and Dobzhansky (1936) that

[1] The research on which this paper is based was done by the author as a student and research assistant at the California Institute of Technology.

both reciprocal crosses with *Drosophila azteca* Sturtevant and Dobzhansky (southwestern United States, Mexico, and Central America) yield hybrids. In previous publications (Miller, 1939, 1941) it was reported that *D. athabasca* males may be made to hybridize with females of *D. algonquin* Sturtevant and Dobzhansky and *D. affinis* Sturtevant (both are eastern American species overlapping the range of *D. athabasca;* they are distributed from the western Great Plains to the Atlantic coast, with *affinis* extending farther to the southeast than *algonquin*).

This paper is a presentation of additional data concerning the hybridization of *D. athabasca* males with females of *D. algonquin* and *D. affinis.*

Hybridization Between *D. algonquin* Females and *D. athabasca* Males

Hybrids were first obtained between *D. algonquin* females and *D. athabasca* males from several crosses involving wild type strains of these species. These offspring, though quite normal in general appearance, were sufficiently different from individuals of the female parent's species to raise the suspicion that they were hybrids and not the result of non-virginity. The supposition that hybrids might be obtained in this combination was confirmed by a cross between *D. algonquin* females homozygous for the recessive mutant gene rough (eyes) and wild type *D. athabasca* males of a Grey's River (Wyoming) strain. The offspring of this cross were wild type with respect to eye character.

Continued interspecific matings have shown that hybrids between *algonquin* and *athabasca* are rather difficult to obtain. In Table 1 are given insemination frequencies involving certain mutant strains of these species. (Recessive mutant strains were usually used as a source of females employed in interspecific crosses so as to have a check on the authenticity of any hybrids produced.) To obtain these data, females and males (about ten of each) were kept together in culture bottles for ten or eleven days, and at the end of this time the females were dissected and their semi-

nal receptacles examined for sperms. Frequencies of inseminated females are presented here as fractions, the denominators of which represent total numbers of females examined. As is shown in Table 1, interspecific insemination was very low in the combination of *algonquin* females and *athabasca* males and was not detected at all in the reciprocal combination.

Hybrids between *algonquin* and *athabasca* have been obtained principally from a series of group matings in each of which five *algonquin* females were kept with five *athabasca* males for about a month (longer if hybrids were being

TABLE 1

FREQUENCIES OF FEMALES FOUND INSEMINATED AT THE END OF THE 10–11-DAY MATING PERIOD

Mutant strains used here were: *affinis* rugose eyes (rug); *algonquin* droop wings (dr) and cinnabar eyes (cn); and *athabasca* vermilion eyes (v) (sex-linked) and cinnabar eyes (cn).

Intraspecific matings:		
affinis ♀♀ × *affinis* ♂♂	("rug")	50/51 (98.0%)
algonquin ♀♀ × *algonquin* ♂♂	("dr")	45/51 (88.2%)
athabasca ♀♀ × *athabasca* ♂♂	("v")	45/53 (84.9%)

Interspecific matings involving *D. athabasca*:

algonquin ♀♀ × *athabasca* ♂♂		*affinis* ♀♀ × *athabasca* ♂♂	
"dr" × "v"	3/53	"rug" × "v"	56/82
"dr" × "cn"	0/54	"rug" × "cn"	34/54
"cn" × "v"	0/25		
	3/132		90/136
	(2.3%)		(66.2%)
athabasca ♀♀ × *algonquin* ♂♂		*athabasca* ♀♀ × *affinis* ♂♂	
"v" × "dr"	0/104	"v" × "rug"	0/102
"v" × "cn"	0/36	"cn" × "rug"	0/24
	0/140		0/126

produced in this time). Various strains of both species were used in these crosses. The *algonquin* females were all from mutant strains; the following recessive characters were used singly and in combinations: dusky wings (dy) and swollen tarsi (sw), both sex-linked; brown eyes (bw) and droop wings (dr), linked to each other on one of the autosomes; and cinnabar eyes (cn) and rough eyes (ro) carried separately on two of the other autosomes. Males were taken from the following wild type *athabasca* strains:

Gravina (Alaska), two strains from Grey's River (Wyoming), Sundance (Wyoming), and Quinault (Washington). The *athabasca* mutant strain vermilion eyes (v) (sex-linked recessive) was also used.

Of 342 such group matings 13 (3.8 per cent.) yielded adult hybrids. All the mutant genes used in the successful crosses (*algonquin* dy, dr, cn, and ro; *athabasca* v) proved to be recessive in the hybrids. The total numbers of hybrids obtained from these crosses were 217 females and 178 males. In 11 of the 13 cases, the sex ratio was about 1 : 1 (totaling 150 females and 154 males), but there was a marked excess of females in two cases (totaling 67 females and 24 males). The reason for these aberrant progenies was not investigated, but it is possible that otherwise unnoticed sex-linked lethal genes were responsible. Since the crosses were group matings, it was not possible to determine whether or not the hybrids derived from a cross were all the offspring of the same two parents, although considering the low frequency of interspecific insemination, it seems likely that in each case they were. The numbers of adult hybrids obtained in the 13 cases ranged from 1 to 77, with an average number of 25.1, which seems low in comparison to *algonquin* pair matings kept under these conditions.

D. algonquin and *D. athabasca* are very similar morphologically. However, certain differences between them are noticeable. As pointed out in the descriptions of these species (Sturtevant and Dobzhansky, 1936), *algonquin* is more brownish than the other *affinis* subgroup species, while *athabasca* tends to be dark brown. With respect to color the hybrids may be described as intermediate to the parent species. Another striking difference concerns the sex combs of the males: *athabasca* males have very small sex combs (4 teeth) while *algonquin* males have rather large ones (8–10 teeth). Although the sex combs of hybrid males broadly overlapped those of *algonquin* males in number of teeth, the average number of sex comb teeth was lower in hybrids than in *algonquin* (6.7 ± 0.073 in hybrids and 7.4 ± 0.072 in *algonquin;* see Fig. 1, A and B). The hy-

brids of both sexes were usually quite normal in general appearance and adult viability. However, from some of the early crosses involving wild type strains (*e.g., algonquin* from Aldrich, Texas, to *athabasca* from Sundance, Wyoming) there were obtained a few females with large wing cells and coarse compound eyes, suggesting polyploidy. An ovary smear from one of these showed oogonia with definitely more than the diploid number of chromosomes characteristic of both these species (ten), but an accurate chromosome count could not be made. In the later crosses such females did not appear.

In order to obtain evidence on fertility, dissections and examinations of hybrid gonads and genital tracts were made. The ovaries of the females seemed quite normal in every respect. Backcrosses of these females to *algonquin* males have shown that the female hybrids are in some cases fertile. These crosses were made as pair matings (hybrid female to male of *algonquin* parent strain) lasting about a month. Altogether 128 such backcrosses were made, and 46 of them (35.9 per cent.) produced adult offspring. The total numbers of backcross flies obtained were 378 females and 295 males (there was an excess of females in all groups of crosses), with a mean family size of 14.4, which is well below that for the crosses that produced the hybrids. Since mutant genes were involved, both mutant and wild type individuals appeared in the backcross progeny. In addition, a few of the backcross flies, both mutant and wild type, exhibited various abnormalities (rough eyes, missing or reduced bristles, wings held out, abnormal abdomen) which were not found in the hybrids. These abnormalities were not noticed early in the backcross counts, and a number of abnormal individuals were undoubtedly overlooked, but altogether 13 abnormal females (3.4 per cent.) and 23 abnormal males (7.8 per cent.) were recognized.

The testes of the male hybrids were found to be somewhat smaller than those of either species, although the rest of the male genitalia appeared to be normal. Smear preparations of hybrid testes revealed no sperms. Altogether no

extensive crossing experiment has been performed with hybrid males, it seems very likely that they are regularly sterile.

Among backcross males there was greater variation in the number of sex comb teeth than there was in the hybrids or in either of the parent species. The number of sex comb teeth in the backcross males varied from 4 through 9 (mean was 6.4 ± 0.094), while variation was from 5 to 8 in the hybrids and 4 to 5 and 6 to 9 in *athabasca* and *algonquin*, respectively. Fig. 1, Parts A, B, and C, shows diagrammatically the distributions of numbers of sex comb teeth.

The only sex-linked mutant gene that was involved in successful backcrosses was the *athabasca* mutant vermilion. Unfortunately, the backcrosses involving vermilion also involved the *algonquin* autosomal recessive cinnabar. Both mutant genes produce bright red eyes. However, *athabasca* vermilion produced regularly a more striking deviation from wild type than *algonquin* cinnabar, which allows for a certain amount of darkening with age. In the backcrosses involving both these mutant genes it was possible to distinguish between two kinds of males with bright red eyes, one definitely more extreme than the other. The extreme type has been called vermilion, and the less extreme one, which was similar to the bright red eye class found in the females, has been called cinnabar. The kinds of offspring of these crosses are given in Table 2, A. According to expectation, the two kinds of females, wild type and cinnabar, were of equal frequencies. However, the frequencies of the classes of males deviated from expectations based on the inheritance of these mutant genes and the assumption that they would together produce bright red eyes. According to these expectations, the males with bright red eyes should have been about three times as numerous as the wild type males. Actually, the vermilion and cinnabar males taken together were only about twice as numerous (deviation significant by Chi-square test). In other backcrosses involving cinnabar (but no other mutant) there was no great deviation from a 1 : 1 ratio of wild type to cinnabar males (Table 2, B;

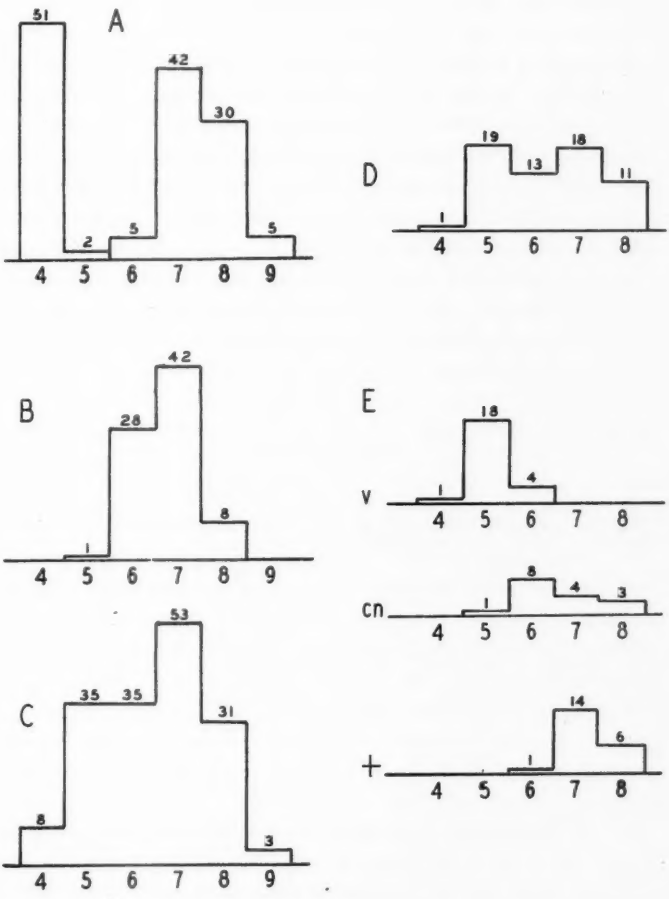

FIG. 1. Histograms representing sex comb teeth counts in *D. algonquin*, *D. athabasca*, hybrids between these species, and males of the backcross of hybrid females to *D. algonquin*. The abscissa classes represent numbers of teeth in the sex comb on the right prothoracic leg. The number at the top of each column is the number of individuals in the class. A. The histogram at the left is based on *athabasca* vermilion males, the one on the right on *algonquin* cinnabar males. B. Hybrids involving the *algonquin* cinnabar strain. C. Backcross males, the *algonquin* strain being cinnabar. D. Part of the males of "C" derived from hybrids containing *athabasca* vermilion. E. The types constituting the backcross males of "D."

deviation not significant by Chi-square test). Consequently, it appears that the presence of vermilion is most probably responsible for the low frequency of males with bright red eyes in the backcrosses involving vermilion and cinnabar. The possibility exists that males carrying *athabasca* vermilion and homozygous for *algonquin* cinnabar die early and were not observed. If this were the case, the backcross ratio of wild to vermilion to cinnabar males should be 1 : 1 : 1, and that, in fact, was the ratio closely approximated by the observations (deviation not significant by Chi-square test). However, since the possibility still exists that vermilion is epistatic to cinnabar and has a semi-lethal effect

TABLE 2

PROGENIES OF BACKCROSSES TO *algonquin* MALES OF *algonquin-athabasca* FEMALE HYBRIDS

A. Offspring of backcrosses of *algonquin-athabasca* females heterozygous for *athabasca* vermilion and *algonquin* cinnabar to *algonquin* males homozygous for cinnabar (totals of three pair matings).

Males			*Females*		
"wild"	"bright red eyes"		"wild"	"cn"	Total
	"v"	"cn"			
27	24	25	47	47	170

B. Progeny of backcrosses of *algonquin-athabasca* females heterozygous for *algonquin* cinnabar (and no other mutant genes) to *algonquin* males homozygous for cinnabar (totals of 24 pair matings).

Males		*Females*		
"wild"	"cn"	"wild"	"cn"	Total
73	67	89	75	304

both with and without cinnabar, and such a relationship might also give the observed results, the lethality of the vermilion-cinnabar combination is not proved. It has been shown by Dobzhansky (1941) that certain mutant genes of *D. pseudoobscura* which do not ordinarily reduce viability appreciably may be quite detrimental in backcross individuals resulting from hybrids between *pseudoobscura* and *persimilis*.

The distribution of sex comb size in the backcross males derived from the cinnabar-vermilion crosses is presented in Fig. 1, D, while in Fig. 1, E, are given the distributions for

the three phenotypic classes separately. It is apparent that the males of the vermilion phenotype, which must have received the *athabasca* X chromosome, tended to have small sex combs (average was 5.1 ± 0.094). (Although counts were not made, the impression of a tendency towards small sex combs was not gotten from the other backcross classes.) A possible explanation for this is that the males with the *athabasca* X were in possession of some of the important *athabasca* genes responsible for the difference in sex comb size between *athabasca* and *algonquin*. Although this may be true, there is also the possibility that in these backcross males small sex combs have developed in response to a peculiar combination of *algonquin* and *athabasca* genetic factors, possibly to the influence of the vermilion gene itself.

Hybridization Between *D. affinis* Females and *D. athabasca* Males

Table 1 also shows insemination frequencies for *D. affinis* and for both reciprocal combinations of *affinis* and *athabasca*. These data were derived in the manner already described for *algonquin* and *athabasca* insemination frequencies. Table 1 shows that the frequency of *affinis* females inseminated by *athabasca* males by the end of the mating period was fairly high (almost two thirds), while the reciprocal combination gave no insemination at all. More recently a few small group matings of *athabasca* vermilion females with *affinis* males of wild-type strains have shown some insemination after ten or eleven days (1/15 for Victoria, Texas; 11/19 for Gatlinburg, Tennessee; and 2/19 for Baltimore, Maryland). Hybrids have been produced by the combination of *affinis* females by *athabasca* males, but to date none of the matings of *athabasca* females to *affinis* males has yielded offspring. It seems likely that more extensive use of wild-type strains of *affinis* will allow for appreciably high frequencies of interspecific insemination of *athabasca* females by *affinis* males and, perhaps, even hybrid production. On the other hand, employment of wild-type *affinis* females and *athabasca* males may well give

reductions in interspecific insemination frequency below those shown in Table 1 (as in series of mass matings reported below).

Some of the crosses made to show insemination frequencies of *affinis* females by *athabasca* males yielded offspring, and these were wild type with respect to eye character, indicating that the normal allele of *affinis* rugose is present in *athabasca*. Other *affinis* recessive mutant genes that have proven to be recessive in hybrids are: cut, pinkish, net, tiny bristle, veinlet, and white (for descriptions, see Sturtevant, 1940).

Although the frequency of inseminated females among *affinis* females that have been kept with *athabasca* males ten or eleven days is fairly high, only very small numbers of hybrids have been obtained. Not only has the frequency of crosses producing offspring been low, but the number of offspring per successful cross has always been very small. The difficulty in obtaining many hybrids from this cross is apparent in the results of a series of mass matings of *affinis* females and *athabasca* males in which the number of flies of each sex (about equal numbers of each) varied from 8 to 15. Here the females were aged 3 to 4 days before mating, and the mating period lasted 10 to 12 days. The *affinis* females were of the following kinds: rugose, Woods Hole (Massachusetts), and strain hybrids of the cross Woods Hole by Austin (Texas). The *athabasca* males were of the following kinds: Sundance (Wyoming), Jackson Hole (Wyoming), Quinault (Washington), Clingman's Dome (North Carolina), and strain hybrids of the cross Jackson Hole by Quinault. Altogether 20 such matings were made. At the ends of the cohabitation periods 201 females were recovered and dissected, and in 51 of these (25.3 per cent.) sperms were found (in 14 out of the 20 crosses at least one inseminated female was found). (Out of 70 *affinis* females kept with *affinis* males under similar conditions 50, or 71.4 per cent., were found to be inseminated.) However, only 6 of the 20 interspecific mass matings yielded hybrids. Moreover, the numbers of hybrids were very low. Alto-

gether there were obtained 13 adult hybrids, 8 females and 5 males. The number of individuals from a successful cross never exceeded 3. The families produced in other crosses of *affinis* females by *athabasca* males have all been of about this size.

D. athabasca and *D. affinis* are somewhat more similar morphologically than *athabasca* and *algonquin*. For example, in both these species the males have small sex combs consisting of about 4 teeth. The hybrids are similar to the parent species and apparently quite normal in all external respects. Since the number of *affinis-athabasca* hybrids has been very small, extensive matings to determine fertility have not been possible. Those matings that have been attempted have not been successful. Smear preparations of testes have revealed no sperms. The ovaries of seven females, each aged at least a week, were found to contain no eggs (*affinis* females may lay eggs in 3 or 4 days after hatching), only clusters of small cells. Thus, there is no evidence that either sex is fertile.

Early in the observations of genital tracts of *affinis* females mated to *athabasca* males the impression was gotten that the sperms in the ventral receptacle were fewer and less active than following intraspecific inseminations. In addition, a certain amount of granular material was frequently present in the sperm masses, and it seemed that this was characteristic of the interspecific combination. In order to check on these impressions, an attempt was made to classify cases of insemination with respect to amount of sperms, activity of sperms, and presence or absence of granular material. Classification was applied to part of the cases of insemination found in the females of the 20 interspecific mass matings mentioned above and also to a number of cases of intraspecifically inseminated *affinis* females. Arbitrary separations were made of cases of "many," "medium," and "few" sperms and separations were also made according to whether the sperms were "active" or "quiet." The results of this classification are presented in Table 3. It may be seen from this table that amounts of

sperms were generally greater following intraspecific cohabitation than after interspecific mating. Also, the frequency of definitely active sperms was appreciably higher following the intraspecific combination. However, granular material in the ventral receptacle was found following both kinds of matings, with almost as high a frequency in the intraspecific combination as in the interspecific one.

The appearance of the sperms in the genital tracts of *affinis* females inseminated by *athabasca* males suggests both fewness of sperms and inactivation of sperms as reasons for the low fertility of these interspecifically inseminated females. It is possible that gametic cross-incompati-

TABLE 3

CLASSIFICATION OF SPERM MASSES OBSERVED IN VENTRAL RECEPTACLES OF *D. affinis* FEMALES AT END OF 10–12-DAY MATING PERIOD

	A. Classification according to quantity:			
	"Many"	"Medium"	"Few"	Total
aff. ♀♀ × *aff.* ♂♂	49 (62.8%)	28 (35.9%)	1 (1.3%)	78
aff. ♀♀ × *ath.* ♂♂	5 (8.9%)	14 (25.0%)	37 (66.1%)	56
	B. Classification according to activity of sperms:			
	"Active"		"Quiet"	Total
aff. ♀♀ × *aff.* ♂♂	76 (97.4%)		2 (2.6%)	78
aff. ♀♀ × *ath.* ♂♂	38 (67.9%)		18 (32.1%)	56
	C. Presence or absence of granular materiál:			
	Present		Absent	Total
aff. ♀♀ × *aff.* ♂♂	37 (47.4%)		41 (52.6%)	78
aff. ♀♀ × *ath.* ♂♂	29 (51.8%)		27 (48.2%)	56

bility, such has has been described for various combinations of Drosophila species (see Paterson, 1942, 1947), is responsible for the state of the sperms following insemination of *affinis* females by *athabasca* males. However, additional study will need to be done to establish the nature of the mechanism.

ACKNOWLEDGMENTS

The author wishes to express appreciation to Professor A. H. Sturtevant, of the California Institute of Technology, and to Professor Th. Dobzhansky, of Columbia University, for Drosophila stocks used in this study and for advice and criticism.

SUMMARY

Drosophila athabasca males will inseminate females of the similar species *D. affinis* and *D. algonquin*, and both these crosses lead to hybrid production. *D. athabasca* females have been inseminated by *D. affinis* males, but hybrids have not been produced.

Female hybrids between *algonquin* and *athabasca* are fertile and have been crossed to *algonquin* males. Sex comb size, which differs between these species (*algonquin* has large sex combs, *athabasca* small ones) has been studied in hybrid and backcross males from some of the crosses.

Although the frequency of *affinis* females inseminated by *athabasca* males is fairly high, the number of hybrids produced by this combination is very low. Neither sex of the hybrids appears to be fertile.

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DISTRIBUTION AND SPECIATION OF THE AMPHIBIANS OF THE RIU KIU ISLANDS

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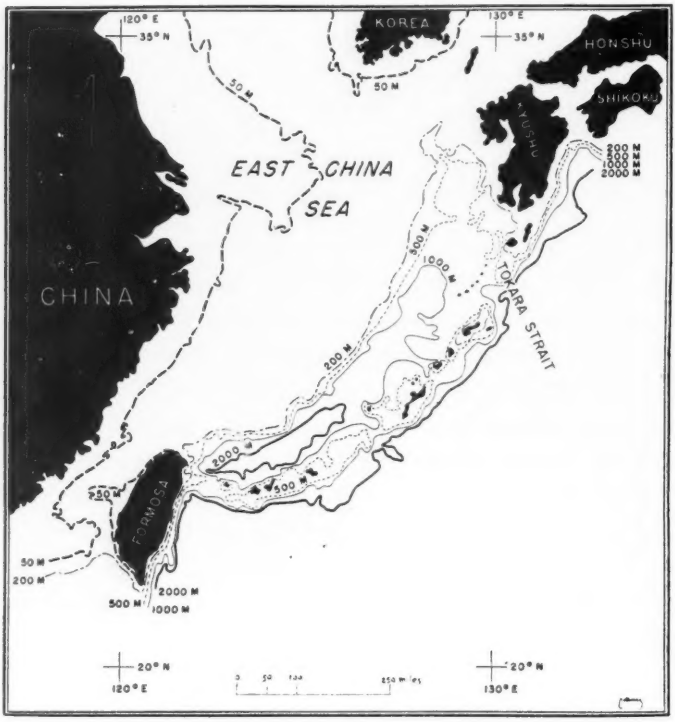
A preliminary survey of the amphibian fauna of the Riu Kiu Islands indicates that these islands constitute an almost perfect natural laboratory for the study of the recent evolution of certain groups of animals. The material that I have examined is from the collections of the California Academy of Sciences, the Chicago Natural History Museum and the United States National Museum. To the authorities of these institutions I am indebted for the loan of material. To Messrs. Karl P. Schmidt and Clifford H. Pope, of the Chicago Natural History Museum, I am further indebted for the use of laboratory space and much helpful advice and criticism.

Geography of the Islands

The Riu Kiu Islands extend in an arc, convex southeastward, from south of Kyushu to a point northeast of Formosa (see Map A). The numerous islands and islets (about 95) that make up the chain have been divided into groups by Tokunaga (1901) and subsequent geographers. These groups together with some of the principal islands[1] of each are as follows, with the groups (after Hanawa, 1935) in geographic order from north to south:

[1] The Japanese spelling of these islands includes the suffix "-shima" or "-jima."



MAP A

Tokara (Linshoten) Group
- Takara
- Kotakara

Oshima Group
- Amami
- Kikaiga
- Tokuno
- Okierabu

Okinawa Group
- Okinawa
- Ie
- Iheya

Okinawa Group (*Continued*)
- Kume
- Ke
- Tokashiki

Miyako Group
- Miyako
- Irabu

Yaeyama Group
- Ishigaki
- Iriomote
- Hateruna
- Yonaguni

Map B shows these islands in greater detail. Hanzawa included still another group, the Osumi, lying just south of Kyushu. For reasons given below I do not consider that the islands of the Osumi Group are part of the Riu Kius.

MAP B

The topography and geology of the region have been worked out in some detail by Koto (1897) and Hanzawa. The account which follows is based largely upon these two authors, supplemented by the correlative work of Yabe and Aoki (1923). The islands may be divided into two curves.

The inner, or western, curve comprising the Tokara Group, Kume, and several small islands lying to the northeast of Formosa, is of volcanic origin. These islands rise out of the trough at the eastern rim of the East China Sea. The outer curve includes all the other islands. These are made up of Proterozoic, Paleozoic, undated igneous rocks, or Tertiary and younger rocks. In any case the islands of the outer curve are remnants of a Riu Kiu Cordillera.

The different groups are separated from one another by sea basins from 400 to 2,000 m. in depth. The deepest of these, the Tokara Strait, lies between Amami and the Osumi Group. The Osumi Group is but 40 km. south of Kyushu and lies on the same submarine platform. The maximum depth of the channel between Kyushu and the Osumi Group, 200 m., is considerably less than that of the Tokara Strait. Thus, on the basis of topography, the Osumi Group is more closely related to the Japanese Islands than to the Riu Kius. This relationship is emphasized by the fauna, as will be shown. The Okinawa Group is separated from the Oshima Group by a basin 800 m. deep, and from the Miyako by one slightly under 1,000 m. deep. The Miyako Group is divided from the Yaeyama by a strait only 400 m. in depth. Formosa and the Yaeyama Group are separated by a basin reaching 800 m. The trough out of which the volcanic islands of the Tokara Group rise is 800 m. deep and is directed NNE and SSW between the Tokaras and Amami. According to Koto, these straits, with the exception of the last, are developed across the strike of the Paleozoic rocks of the cordillera and are probably the result of faults.

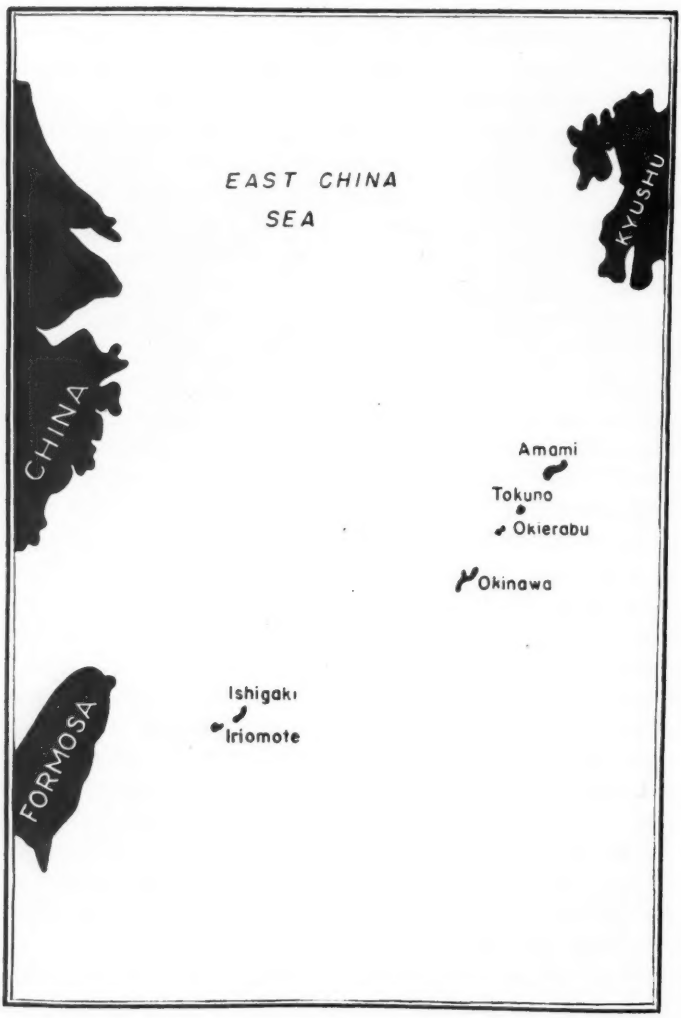
The islands vary considerably in size, the largest, Okinawa, being 110 km. and 20 km. in its greatest dimensions. The maximum length and width of Amami are 57 km. and 30 km., of Miyako 25 and 15, of Ishigaki 35 and 20. The smallest islets are less than one square kilometer in area. In relief the islands also show much variation. Some, like Miyako, are relatively flat with one or two low ridges. Iriomote consists of many flat-topped ridges. Ishigaki and

Okinawa are mountainous in parts and relatively flat in others. The greatest elevation is 690 m. on Amami.

Geologic History

The Riu Kiu Cordillera arose in the late Permian or early Mesozoic. The history of the cordillera between the Permian and the Upper Eocene is uncertain, as there are no deposits that can be definitely placed in that interval. Nevertheless, it is likely that during that interval the cordillera was broken into a number of mountainous masses by erosion and faulting. Subsequent to the Eocene there have been many fluctuations in sea level great enough to alter radically the area available for habitation by land animals. The entire Miyako Group, Hateruma and Yonaguni in the Yaeyama Group, Kume and most of the lesser islands of the Okinawa Group, and Kikaiga in the Oshima Group seem to have been most subject to inundation in the stages of land submergence (Hanzawa, 1935).

Since the Eocene there have been as many as three periods when the Riu Kius could have had direct land connections with Asia through Formosa: during the Oligocene, the late Pliocene and the earliest Pleistocene. The actual extent of land in the Oligocene emergence is imperfectly known (Hanzawa, 1935). At the height of the Pliocene emergence, sea bottom that is now at a depth of 700 m. was raised above sea level (Yabe, 1929, 1929a). Thus during the late Pliocene the distance between the continent and the center of the Riu Kiu curve could not have exceeded 70 miles. This figure was determined by measuring the distance between the 500 m. submarine contours on Map A. Hanzawa has stated that during the Pleistocene land emergence the Oshima and Okinawa Groups were connected, as were the Miyako and Yaeyama Groups. This would imply an emergence as great as that of the Pliocene unless the channels now separating the groups were not so deep in the Pleistocene. Hanzawa did state that in all probability the latest tectonic movements have given the channels their present depth. Given Hanzawa's conditions, the distance



MAP C. Probable land area of the Riu Kiu Islands at the climax of the land submergence between the Oligocene and Pliocene. Present areas of China, Formosa and Kyushu shown in black.

over water between the Yaeyama Group and Formosa would have been negligible.

Invasion of the land by the sea was greatest during the interval between the Oligocene and Pliocene. Only the highest points in the islands remained above sea level (Hanzawa, 1935). The inundation of the land between the late Pliocene and early Pleistocene stages of emergence was almost of equal extent. There have been further minor fluctuations in sea level since the early Pleistocene. In one of these the larger islands such as Amami, Okinawa and Ishigaki were connected with their dependent islets. Maps C and D illustrate stages in the history of the cordillera.

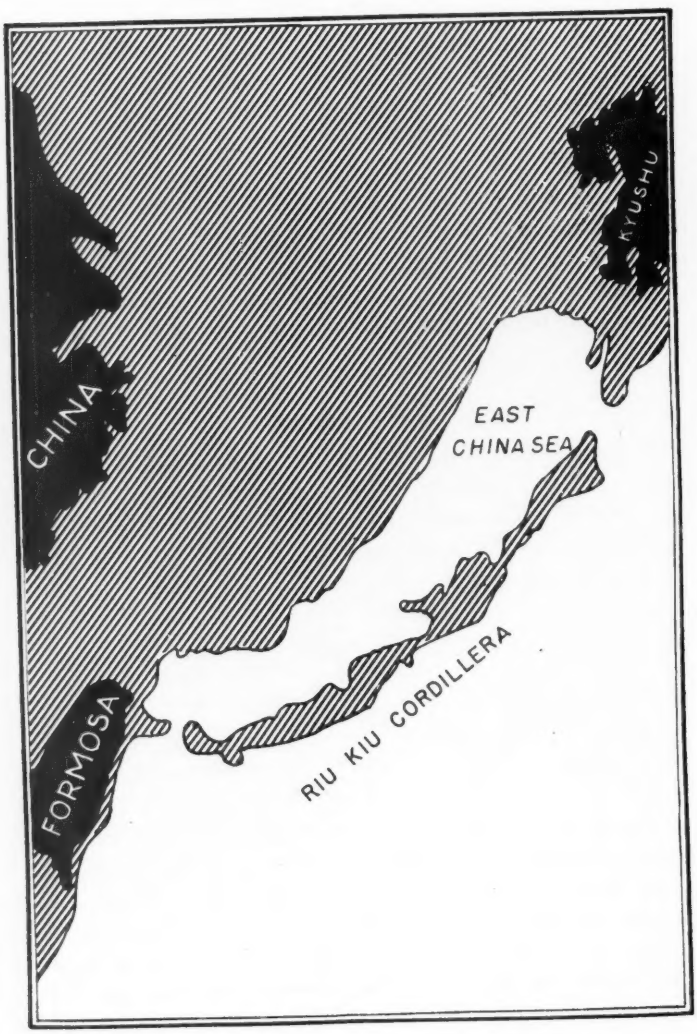
Faunal Analysis

ORIENTAL ELEMENTS

The amphibian fauna of the Riu Kius is made up primarily of two elements: endemic forms (derived presumably from Oriental species) and Oriental species that reach the islands without changes regarded as of specific order. The following species are part of the general Oriental fauna:

Salientia:	Distribution in Riu Kiu Groups:
Microhyla ornata	all groups except Tokara Group
Rana limnocharis	" " " " "
Rhacophorus schlegelii	" " " " "
Rhacophorus eiffingeri	Oshima, Okinawa and Yaeyama Groups
Rhacophorus japonicus	all groups

Microhyla ornata is found in southern China, Formosa and the peninsula of southeastern Asia as well as in the Riu Kius. *Rana limnocharis*, generally distributed in the East Indies, the peninsula of southeastern Asia, Formosa and China, is one of the few Oriental species reaching Japan. Outside of the Riu Kius *Rhacophorus japonicus* has been reported from Formosa only. Though *R. schlegelii* and *R. eiffingeri* either occur in Japan or are closely related to Japanese forms, the distribution of the genus is southern, being found in the East Indies, southeastern Asia and Madagascar.



MAP D. Probable maximum extension of land in the late Pliocene. Present areas of China, Formosa and Kyushu shown in black.

ENDEMIC ELEMENTS

Approximately one half of the amphibian species of the Riu Kius are endemic. They are:

	Distribution in Riu Kiu Groups:
Caudata:	
Triturus ensicaudus	Oshima and Okinawa Groups
Tylototriton andersoni	Okinawa Group
Salientia:	
Hyla hallowelli	Oshima Group
Rana holsti	Okinawa Group
Rana subaspera	Oshima Group
Rana ishikawae	Okinawa Group
Rana narina	Okinawa and Yaeyama Groups
Rana namiyei	Okinawa Group
Rana okinavana	Okinawa and Yaeyama Groups

Of these, five show affinities to contemporary Oriental species. Only four other species of *Tylototriton* are known. They are from isolated localities in southern and western China. *Hyla hallowelli* is similar to *chinensis* of Formosa (Van Denburgh, 1912) and *Rana namiyei* to *kuhlii* of Formosa and southern China. Stejneger (1907) cites the relationships, which are open to question, of *Rana okinavana* to *lateralis* of southeastern Asia and of *R. narina* to *everetti* of the Philippines. *Triturus ensicaudus* is closely related to *T. pyrrhogaster* of Japan. The genus is Holarctic in distribution. The remaining three species, *R. subaspera, R. holsti* and *R. ishikawae,* do not have any clearly defined geographic relationships.

The extent of endemism in the fauna of the islands is reflected in the inter-island differences of species endemic to the Riu Kiu chain, and even in the more widespread, relatively undifferentiated forms. Illustrative of this is the local differentiation of *Rhacophorus schlegelii.* In this species the populations on the various groups of islands differ with respect to coloration, texture of the skin and body proportions. Fig. 1 shows the variations in the pattern on the posterior surface of the thigh from one island group to another. In Table I are listed the differences in body proportions.

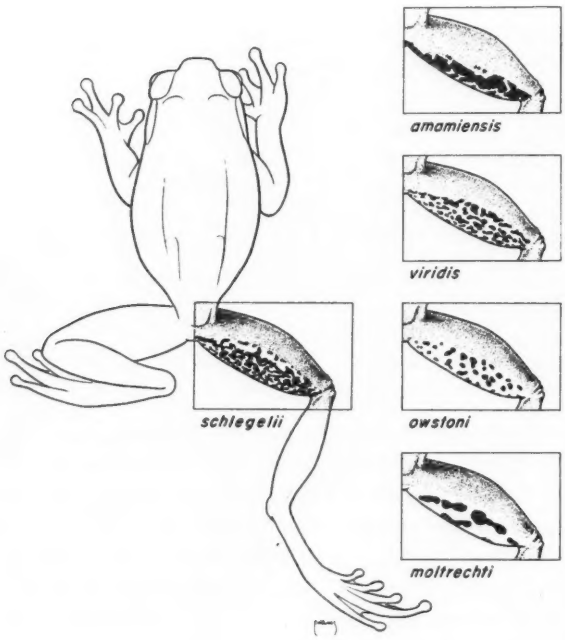


FIG. 1. Geographic variation in the color pattern in subspecies of *Rhacophorus schlegelii*. The subspecies are arranged in geographic order from north to south. The typical form is found in Japan, *amamiensis* in the Oshima Group, *viridis* in the Okinawa Group, *owstoni* in the Yaeyama Group and *moltrechti* in Formosa.

TABLE 1

DIFFERENCES IN BODY PROPORTIONS OF AMAMI, OKINAWA AND ISHIGAKI POPULATION OF *Rhacophorus schlegelii*

	Amami *schlegelii amamiensis*				Okinawa *schlegelii viridis*				Ishigaki *schlegelii owstoni*
No.	14				35				45
		Diff.	t	P		Diff.	t	P	
Body length / Leg length	.666 ± .008	.001	0.175	.86	.667 ± .004	.012	2.294	.03	.679 ± .003
Tibia / Leg length	.296 ± .001	.006	2.940	.007	.302 ± .001	.008	5.574	.001	.294 ± .001
Head width / Body length	.348 ± .003	.003	0.672	.51	.351 ± .003	.026	7.239	.001	.377 ± .002

Rana limnocharis presents an example of a widespread species that has not speciated in the islands yet shows a

considerable amount of inter-island variation. Table II lists the differences in body proportions that I have observed in this species.

TABLE II

DIFFERENCES IN SIZE AND BODY PROPORTIONS OF OKINAWA AND ISHIGAKI POPULATIONS OF *Rana limnocharis*

	Sex	Okinawa	Ishigaki	Difference	t	n	P
Log body length	♀	1.5919 ± .0133	1.7089 ± .0034	.1170	5.005	19	.001
Log body length	♂	1.5509 ± .0087	1.6874 ± .0064	.1365	11.550	27	.001
Body length / Leg length	♀ + ♂	.657 ± .008	.635 ± .005	.022	1.954	49	.056
Tibia / Leg length	♀ + ♂	.284 ± .001	.297 ± .002	.013	5.200	49	.001

The differences, in the case of both *limnocharis* and *schlegelii,* are between populations in different *groups* of islands. In *Hyla hallowelli* we have an example of interspecific variation between populations that are in the same island group. *H. hallowelli* is found on Amami and Kikaiga of the Oshima Group. Inasmuch as Kikaiga is completely overlaid by an early Pleistocene limestone, the *hallowelli* population on that island must have become separated from the Amami population not earlier than the early Pleistocene. In Table III are presented the differences in body proportions and size of these two populations.

TABLE III

DIFFERENCES IN SIZE AND BODY PROPORTIONS OF AMAMI AND KIKAIGA POPULATIONS OF *Hyla hallowelli*

	Amami *hallowelli schmidti*	Kikaiga *hallowelli hallowelli*	Difference	t	n	P
Log body length	1.5032 ± .0041	1.4827 ± .0033	.0205	3.727	42	.001
Tibia / Leg length	.301 ± .002	.313 ± .001	.012	5.435	42	.001
Head width / Body length	.315 ± .002	.336 ± .003	.021	4.878	42	.001

The same intra-specific variations appear in the salamander, *Triturus ensicaudus.* There are two types of light markings found on the dorsum in this species: a spotting limited to the mid-dorsal region, and a dorso-lateral line. Table IV shows the differences in marking of samples from Okinawa and Amami.

Using a 2×2 contingency table for each type of marking, chi-square, in the case of dorsal spotting, was found to

TABLE IV

DIFFERENCES IN COLOR PATTERN BETWEEN OKINAWA AND AMAMI POPULATIONS OF *Triturus ensicaudus*

	Okinawa	Amami
Specimens showing dorsal spotting	75	1
Specimens showing dorso-lateral line	30	24
Total specimens examined	149	51

be 37.74 ($n = 1$, $P = .001$); chi-square in the case of the dorso-lateral line was 13.97 ($n = 1$, $P = .001$). As in the frog species cited above, there were also differences between these two populations in total length and in the ratios $\frac{\text{head width}}{\text{body length}}$ and $\frac{\text{head depth}}{\text{body length}}$.

As stated in the opening paragraph, the Riu Kius form an exceedingly good natural laboratory for the study of speciation. The elongate and narrow range afforded by the chain is essentially one-dimensional with consequent increase in the rate of differentiation (Wright, 1940). The breaking up of the cordillera has of course introduced discontinuities into any previously existing clines and fragmented the populations. Once the isolation produced by the division of the cordillera was established local selection could act more effectively and add to the increase in the rate of differentiation. With the reduction of their size, the fragmented populations would tend to show the drift effect (Wright, 1940) with non-adaptive added to adaptive differentiation.

Although at present I am unable to demonstrate the adaptive nature of any inter-island variation, it is not unreasonable to expect that further intensive investigation will reveal such adaptive differentiation. In *Rana holsti* and *R. subaspera* the metacarpal spine (not found in other Asiatic ranids) presents a case in which differentiation, presumably originating in the islands, has been adaptive. The males of these closely related species use the metacarpal spine as an aid in maintaining the grip on the females when mating. Evidently the very tough skin (for frogs) protects

the female during amplexus. As an example of non-adaptive modification, I offer the syndactylism found in the salamander, *Tylototriton andersoni*. Of 40 specimens examined, 6 have one or more syndactylous feet. I do not know whether this condition is the primary one in these individuals or has resulted from faulty regeneration. However, in either event the character must have a genetic basis. None of the 200 specimens I have seen of *Triturus ensicaudus* has a syndactylous foot although presumably there are some cases of regeneration in a sample of that size.

Another very interesting feature of *andersoni* is that the number of toes is variable. Species of the genus *Tylototriton* have either four or five toes. In my sample of *andersoni* every gradation from four to five toes is represented. I have arbitrarily established three classes for types of feet in the series: 4 toes, four metatarsals; 4 + toes, five metatarsals but no phalanges for the fifth; and 5 toes, five metatarsals with one or two phalanges for the fifth. These categories were determined after examination of X-ray plates of the forty specimens. After eliminating the seven syndactylous feet, which could not be placed in this classification, the distribution was three with toes 4, twenty with toes 4 +, and forty-five with toes 5. It should be emphasized that there was a smooth transition from the condition with four metatarsals to that with five metatarsals and phalanges for the fifth. In a number of the feet of the 4 + type, the fifth metatarsal was very small. It should also be stated that, although both feet of any individual tended to fall in the same category, there were combinations of 5 toes with 4 + toes and 4 + toes with 4 toes: syndactylous feet appeared in combination with all three categories. It is possible that the sample was taken when the population was drifting from one condition, say 5 toes, to another, 4 toes. As the series I have examined was collected in 1910, it should be interesting to compare it with a new sample to determine whether any shift in the population had been made since that date.

PALAEARCTIC ELEMENTS

Bufo bufo miyakonis, with its distribution limited to Miyako, is the only strictly Palaearctic form in the Riu Kius. The presence of this toad on only one island and its similarity to *B. b. gargarizans* of China indicate that it may be a recent introduction. The fact that it is restricted to Miyako eliminates the possibility of its being the remnant of a form once widely distributed in the archipelago; for, as I have pointed out earlier, Miyako has been subject to complete inundation and has been, therefore, an entirely unsatisfactory habitat over a long period of time. The distinction of *miyakonis* from *gargarizans* has not, in fact, been supported by any evidence other than geographic isolation.

A paragraph about the fauna of the Osumi Group (not considered part of the Riu Kius) is in order at this point. In these islands are found such palaearctic forms as *Hyla arborea japonica, Rana japonica, R. nigromaculata, R. rugosa* and *R. temporaria.* All these frogs occur in Japan but none south of the Tokara Strait. None of the species found in the Riu Kius (as here restricted) has been recorded from the Osumi Group, even though two are found in Japan. The split in the frog fauna at the Tokara Strait is parallel to the separation of Palaearctic and Oriental mammalian faunas. This faunal division, known as Watase's Line (Okada, 1931) coincides with the geographic division mentioned previously. Apparently the channel between the Osumis and the Oshima Group has proven a barrier to the southward migration of the northern fauna.

Faunal Origins

The distribution of the non-endemic species and that of the forms allied to the endemic ones indicate a southern origin for the amphibian fauna of the Riu Kius. As stated above there were at least three periods of land emergence when the islands might have been connected with the continent. An examination of the bathymetric data of Map A indicates that less change in sea level would be required to link Formosa with the southern Riu Kius than would be

needed to link Japan with the Riu Kius. Thus Formosa is a more probable "bridge" to the archipelago than is Japan.

An alternative mode of dispersal, by no means exclusive of emigration by land from Formosa, is accidental transportation, or waifing, on drifting masses of vegetation. If this method of dispersal is utilized at all by Amphibia, it is necessarily true that it is of greatest importance where the water gap to be crossed is the least (see Darlington, 1938, and Mayr, 1944, for further discussion of this point). On Map D is shown the probable maximum extension of land during the Pliocene. At this stage the easternmost points of the continent were not over 70 miles from parts of the Riu Kius. Under this circumstance waifing from the continent becomes likely. It is apparent that waifing from Formosa, if there were no direct land connection, would also be facilitated at this time.

The importance of accidental transportation can be brought out by a comparison of the frog faunas of Japan and the Riu Kius. Such Oriental (and Riu Kiu) species as *Rana limnocharis* and *Rhacophorus schlegelii* are found in Japan. The bulk of the Japanese fauna, however, is composed of Palaearctic forms, for example, *Rana nigromaculata, R. japonica, R. temporaria, Hyla arborea japonica* and *Bufo bufo japonicus*. In the coastal provinces of China, *nigromaculata* ranges as far south as Kwangtung, *japonica* as far as Fukien, and *Bufo bufo* as far as Kwangtung and even into Formosa. These ranges, which extend farther south than the Riu Kiu Archipelago, indicate that these species can tolerate the climatic conditions of the Riu Kius. *Rana limnocharis*, in China, is not found north of Shantung, approximately the latitude of southern Honshu. *Rhacophorus schlegelii* does not occur on the continent at all. The question arises as to the manner in which these last two species reached Japan. If they did not enter by way of Korea, and their present range places this avenue in doubt, perhaps they used a land bridge between the northern Riu Kius and the Osumi Group. This suggestion can

be discarded at once because it assumes that the Palaearctic species, which are not present in the archipelago, could not utilize the same land bridge to expand their ranges southward. The only explanation remaining is waifing, that *limnocharis* and *schlegelii* managed to reach Japan by accidental transportation on rafts of vegetation. Again the Palaearctic species of Japan must be considered. The waifing explanation still assumes some selective mechanism that the land bridge hypothesis could not supply. But in this case natural selective mechanisms exist. The ocean currents, prevailing winds and typhoon tracks along the coast of Asia are all directed northward. All three physical factors are obviously related to waifing and all are favorable to the idea presented above. Here, then, is a likely explanation for the significance of Watase's Line to the distribution of amphibians and mammals.

The direction of water and wind currents lends further support to the idea of a southern origin for the frog fauna of the Riu Kiu Islands. The presence of the salamander *Tylototriton* in the archipelago may also be the result of waifing from Formosa or southern China. The form whose distribution seems to defy such an explanation is *Triturus ensicaudus*.

The differences in the distribution of the various forms is of significance to the study of the order in which they reached the archipelago. Generally the endemic forms must be said to have entered the island earlier than those found also on the continent. Forms found on the periphery of continents are frequently primitive. The Riu Kius present an almost ideally peripheral area with respect to Asia. Island forms are also released from some selection pressures to which continental species are subject. Given primitive forms and reduced selection pressure, it follows that the longer an island form is isolated the less able it is to expand its range in the face of immigrations of continental ones. It is also true that the longer a species exists in an elongate and narrow range such as is provided by the Riu Kiu chain the more likely are discontinuities to develop in the distri-

bution. In a chain of islands this will be a direct result of the fluctuations in sea level over a long period of time (see page 99 *ff.*). Therefore, from the preceding observations, an endemic form, found only on certain of the islands, probably has been in the archipelago longer than one found throughout the length of the chain and on the continent as well.

To utilize this idea, I have divided the frog fauna as follows:

Limited dispersal: *Hyla hallowelli; Rana holsti; R. ishikawae, R. namiyei; R. narina; R. okinavana; R. subaspera.*
Moderate dispersal: *Rhacophorus eiffingeri; R. japonicus; R. schlegelii.*
Wide dispersal: *Microhyla ornata; Rana limnocharis.*

By limited dispersal is meant distribution limited to one or two island groups in the Riu Kiu chain. By moderate dispersal is meant dispersal in more than two island groups plus Formosa and/or Japan. The final category, wide dispersal, indicates dispersal throughout the archipelago plus southeastern Asia. If restricted range is an indication of early entry, the "limited" species were in the early wave (or waves) of immigration, whereas the others entered later. I am not prepared to distinguish between the last two groups of species as to date of entry.

It is possible to see the effect of fluctuations in sea level on the distribution. *R. okinavana,* for example, has been recorded from Ishigaki in the south and Okinawa in the north. For this discussion I assume that the localities are correct and that exploration on Miyako will fail to reveal *okinavana* there. From Map B the Miyako Group is seen to lie between Okinawa and Ishigaki. Geologic investigation of the Miyako Group has shown that these islands are completely covered by early Pleistocene limestone formations. Therefore they must have been entirely under water when those rocks were deposited, thus destroying their populations of frogs. If *okinavana* had a continuous distribution in the archipelago before the early Pleistocene, the subsequent changes in sea level would have interrupted that distribution by destroying at least the Miyako population.

The distribution of *R. narina,* found in the Yaeyama and Okinawa Groups, can be explained in the same fashion.

Hyla hallowelli presents a slightly different problem. This species occurs only on Amami and Kikaiga in the Oshima Group. Its similarity to *H. chinensis* of Formosa seems to eliminate the possibility of a Japanese origin. One must assume either that the present population is the remnant of a species formerly widely distributed in the Riu Kius, or that it originally entered the Oshima Group as a waif from eastern Asia or Formosa and has never extended its range. Whichever assumption is true, the presence of *hallowelli* on Kikaiga can not antedate the early Pleistocene because the entire island is covered with a Pleistocene limestone, indicating complete submergence. However, the date of entry into Amami can not be so easily determined. Much depends on which of the assumptions is correct. It should be pointed out that the chances for waifing directly from the continent have been reduced since the Pliocene, when the water gap was narrowest.

The question of vagility can not be ignored in the preceding deductions. Waifing must be a function of the size of the animal and of the size of the population from which it originates. Therefore such forms as *Rana limnocharis, Microhyla ornata* and *Rhacophorus japonicus* must possess a great deal of vagility as compared to the large *Rana namiyei,* which exists in relatively small numbers. It is very likely that the presence of *limnocharis* and *japonicus* in so many islands is as much a result of waifing as of dispersal along land routes. Yet one is immediately struck by the contrast made by the relatively restricted ranges of *Rana okinavana* and *Hyla hallowelli,* which frogs are of approximately the same size as *limnocharis* and *japonicus,* respectively. Another factor in vagility is ecological opportunity. Obviously, to be strictly comparable two species must be similar ecologically. The pairs of species *limnocharis-okinavana* and *japonicus-hallowelli* satisfy this requirement. It might be argued that the differences in extent of range are due merely to vagility and not related

to length of occupancy in the islands. However, vagility is one of the attributes of biological "success." It should be remembered that part of my argument states that the longer a species remains in the islands the less successful it is likely to be. Therefore, if the comparison is of ecologically similar animals, differences in vagility support the hypothesis that the "limited range" species were the first to enter the islands.

The problem of the order of entry into the Riu Kius may be attacked from a slightly different angle. Let us suppose that Species A is found only on Amami and Okinawa, with no closely related form or forms either in other parts of the archipelago or on the continent. Species B is found throughout the islands and on the continent. Assuming that the emigrations of both are relatively limited by water, the last time the Amami and Okinawa populations of both A and B were able to interbreed freely (*i.e.*, Amami A $\times$ Okinawa A; Amami B $\times$ Okinawa B) was at the time of the last connections of the two islands. Suppose that a comparison of the two populations of each species reveals that Species B shows more inter-island variation than A. We may then conclude that B has a more rapid rate of evolution than A. The fact that A has speciated (*i.e.*, diverged farther from its continental allies) despite its slower rate of evolution, whereas B has not indicates that A has been in the islands longer than B.

Again the question of degree of vagility arises. Granting the relatively great vagility of small forms occurring in large numbers, once such a species has become established on any of the larger islands the effect of accidental introductions on the population is slight. It is inconceivable that the amount of waifing per year into any one of the larger islands should exceed one thousandth of one per cent. of the population on that island in the case of a frog like *R. limnocharis*. The number of individuals of *limnocharis* on the larger islands must be very large, judging by its abundance in other parts of its range. Consequently the effect of the introduced individuals would be negligible.

The fact that the effect is not enough to offset the divergence of two separate populations is apparent from the differences observed between the Ishigaki and Okinawa series of *limnocharis* I have examined (see page 104). In those species occurring in such small numbers that introductions would have a definite effect, the opportunities for waifing are reduced because of the small size of the population. Therefore, as concerns this second method of determining the order of entry of the fauna into the islands (*i.e.*, using the criteria of speciation and inter-island variations), differential probabilities for chance introduction are not important.

Conclusion

The purpose of this paper has been to examine the amphibian fauna of the Riu Kiu Islands and to discuss some aspects of the speciation of a constituent species. I believe that certain data, especially the inter-island variation (even within the same group of islands) in a species like *Hyla hallowelli*, indicate the rapidity of evolution in these islands. Historical geology explains the discontinuities in the distributions of species such as *Rana okinavana* and *R. narina*. I have shown that the data suggest the order in which the various elements entered the fauna. The test of the hypothesis concerning the order of entry must wait upon the collection of large samples from all the islands. These samples would also serve the equally important purpose of a comparison of the rates of evolution in the various species. The amphibian fauna plainly exhibits the several stages of species formation under island isolation—undifferentiated widespread forms (*Microhyla ornata*), incipient differentiation in widespread forms (*Rana limnocharis*), subspeciation in some forms (*Hyla hallowelli* and *Rhacophorus schlegelii*), and sharply defined endemic species (*Rana holsti*).

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REPRODUCTION IN PLANTS[1]

RECENT DEVELOPMENTS IN THE CONTROL OF FLOWERING BY PHOTOPERIOD

H. A. BORTHWICK,[2] M. W. PARKER,[2]
AND
S. B. HENDRICKS[3]

INTRODUCTION

THE phenomenon of photoperiodic flowering control has been well described in the thirty years since its discovery (Murneek and Whyte, 1948) and has been found to occur widely, both biologically and geographically. Resolution into constituent parts such as causative mechanism and method of expression, however, has been difficult. Study of light action, which is the major subject of the treatment here, fortunately has opened up many unexpected approaches and now makes a general advance possible.

APPROACHES TO THE PROBLEM

Significant information concerning the control of flowering by photoperiod is derived from plants growing under natural conditions (Samygin, 1946). Closely related plants, such as soybean varieties, exhibit a wide range of responsiveness to variations in length of the natural daily light

[1] Papers from a symposium presented at the meeting of the American Society of Naturalists during the sessions of the American Association for the Advancement of Science at New York, N. Y., before a joint session with the Botanical Society of America, Genetics Society of America, Ecological Society of America, American Society of Plant Physiologists, and American Society of Zoologists, December 30, 1949.

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period or photoperiod (Borthwick and Parker, 1939). All flower promptly when subjected to photoperiods of 8 to 10 hours, but some fail to flower with photoperiods that exceed about 14 hours. Others fail at slightly longer photoperiods, but some flower even in continuous light. Varieties thus range from strictly short-day types to those that are not distinct from indeterminate plants, *i.e.*, plants whose flowering is apparently uninfluenced by variations in photoperiod. Somewhat similar series can be cited among long-day plants. All long-day plants flower in continuous light, but some are able to flower on comparatively short photoperiods. Plants of the latter type are thus similar to indeterminate ones. Short-day, indeterminate, and long-day plants thus appear to form a continuous series, and the reactions that lead to flowering in all are probably similar. The differences in response of long- and short-day plants are possibly due to differences in balance between various reactions in the series that lead to flowering rather than to differences in kinds of reaction. Indeterminate plants are uninfluenced by photoperiod, not because they lack the flower-inducing reactions of both long- and short-day plants, but because of the way in which these reactions are balanced. The intermediate day-length plants described by Allard (1938) would be ones in which reactions are so balanced as to inhibit flowering when photoperiods are either too long or too short. This group includes such plants as *Mikania scandens, Phaseolus polystachys, Eupatorium torreyanum,* and certain wild forms of *Saccharum spontaneum.*

The term photoperiodism is in some respects a misnomer because the time-dependent phases of the reactions that result in flowering of short-day plants and in inhibition of flowering of long-day plants apparently occur during dark periods rather than light periods, and the effect produced on the plant depends on the length of the uninterrupted dark period. A soybean flowers when it receives 8-hour days and 16-hour nights not because the light periods are short but because the dark periods are long. If each dark

period is broken near the middle by a brief period of light flowering fails because the long dark-period has been changed into two short ones neither of which is conducive to flowering. With long-day plants similar treatment results in opposite response. Winter barley, for example, fails to flower if it receives 12-hour photoperiods and 12-hour dark periods; but if the 12-hour dark periods are interrupted near the middle flowering promptly occurs. The " periodism " part of " photoperiodism " should thus be considered as referring to duration of darkness rather than duration of light. The " photo " part of the term refers to photoreactions that occur whenever the plant is irradiated. After a period of darkness such irradiation annuls an action that occurred during the darkness.

Control of flowering, *i.e.*, prevention of flowering of short-day plants and promotion of flowering of long-day plants, can be obtained under optimum conditions by application of white light of very low energy. The magnitude of energy required is easily determined by irradiating plants near the middle of the dark period for a given number of minutes with light of various intensities and finding the minimum amount that is required to inhibit flowering in the short-day plant or promote it in the long-day one. For soybean, energy equivalent to about 10 foot-candle minutes of radiation from an incandescent-filament lamp is adequate (Parker, Hendricks, Borthwick and Scully, 1946). Other plants vary somewhat, but the requirement of each is of this same magnitude (Borthwick, Hendricks and Parker, 1948). It is also significant that the entire range of energy required to pass from no effect to complete control of flowering is approximately five-fold.

Flowering can be controlled through the leaf and the effects transmitted to the meristems where floral differentiation occurs. These reactions can be so greatly reduced in the case of Biloxi soybean by reducing the temperature of the leaf to 50° F. during the long dark periods that flowering does not occur (Parker and Borthwick, 1943). The plant makes normal growth, however, which indicates that

the products of photosynthesis are being moved out of the leaf as usual. Since the new growth consists of vegetative structures it follows that failure of flowering is to be attributed to inadequacy of flower-inducing stimuli at the meristems. Flowering can also be prevented in soybeans by maintaining the petioles at low temperature, thus inhibiting transport of the stimulus through them. The temperature required, however, is near the freezing point, and this also stops movement of photosynthate as well as the flower-regulating materials. The flower-inducing stimulus is evidently transported by living cells, for it is stopped by steam girdling of the petioles of leaves that are receiving short-day treatments (Withrow and Withrow, 1943).

Consideration thus far has been mainly of effects of photoperiod on the initiation of flower primordia, but photoperiod is also involved in the subsequent development of flowers and the maturation of fruits. In this latter respect plants also differ greatly. An exposure of Biloxi soybeans, for example, to five or six 8-hour days and 16-hour nights may result in the formation of flower primordia, but unless further short-day treatment is given these undergo little development and are eventually abscised. A similar or less drastic treatment to cocklebur is adequate to ensure maturation of fruits. The greater responsiveness of cocklebur to treatment may result from any of several possibilities. Cocklebur may, for example, be able to continue generation of the stimulus after the initial treatment is received. Another possibility is that a greater amount of the flower-inducing material is generated by a given treatment. Meristems of cocklebur may require far lower concentration levels, or possibly the flower-inducing substance in cocklebur is less subject to decomposition than in some other plants. These possibilities merely suggest some of the problems that are involved in the developmental effects of photoperiod.

On the basis of information from grafting experiments and other sources many workers have accepted the proposal that a special flower-inducing hormone (Cajlachjan, 1937),

called florigen, is generated in the leaves and that it moves to the meristems where it induces floral differentiation. There is no information as to the possible nature of such a hormone and an assay that will aid in obtaining this information still has to be developed.

Stem elongation is usually associated with floral initiation in many long-day grasses and other long-day species that are characterized by rosette formation under short-day conditions. Changes in coloration of leaves and stems for certain varieties of *Perilla* and of leaves for some soybean varieties as well as bracts in *Poinsettia* and some other species accompany flowering.

Other non-flowering responses of plants to photoperiod are known. The bulbing of onions, for example, occurs under conditions of long photoperiods but fails with short photoperiods (Magruder and Allard, 1937). Garner and Allard in their early work found that abscission of leaves for certain kinds of trees is delayed by supplemental light. Tuberization in such plants as artichoke (Hamner and Long, 1939) and potato (Werner, 1936) has been found to be photoperiodically influenced. The experimental conditions that induce these responses are those that in other plants, and sometimes in the same ones, result in control of flowering. A close relationship as to mechanisms involved is indicated.

Action Spectra for Floral Initiation

Prevention of floral initiation in Biloxi soybean by a brief period of illumination near the middle of each dark period, of optimum length for flowering, permitted a detailed study of the action spectrum (Parker, Hendricks, Borthwick and Scully, 1946). Such a spectrum expresses the energy required for obtaining a specific biological response. In spectrographic work young soybean plants that had been maintained vegetative by long photoperiods, were defoliated to a single leaflet, and placed on a schedule of 10 hours light and 14 hours dark, for six consecutive days. Such plants initiate flower primordia if the dark period is con-

tinuous. If during each dark period, however, the leaflet is irradiated, floral initiation is influenced. The effectiveness of the treatment can be determined by the number of flower primordia formed. Radiation used was obtained by means of a spectrograph and different levels of energy were obtained for each wave-length region by varying the time of exposure with different lots of plants. Following the six-

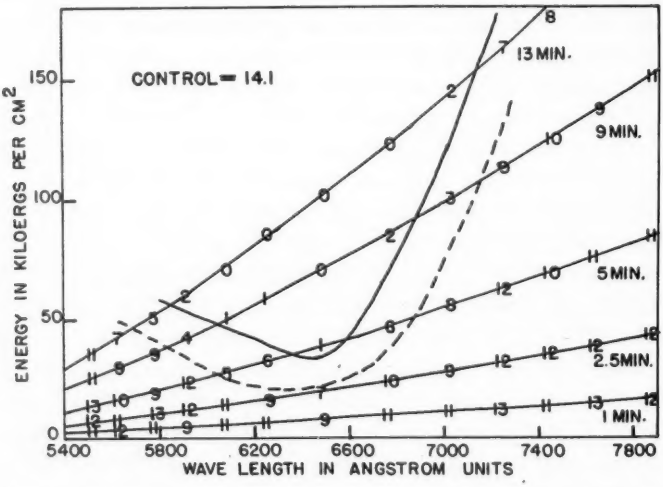


FIG. 1. Response curve for suppression of floral initiation in Biloxi soybean in the red region of the spectrum. Resulting energy at each wave-length station is plotted, and superimposed on energy lines are total numbers of flower primordia formed by lots of four plants which received a specific wave-length band at different energy levels. The curves were drawn freehand through energy lines, crossing between points at which sharp breaks occurred in number of flower primordia produced. The solid curve was taken as the one representing the response, while broken-line curve illustrates gradation effect with decreasing energy.

day treatment plants were returned to long photoperiods for development prior to dissection, which was made fourteen days after the beginning of an experiment. If the flower-forming stimulus is relatively weak, flower primordia are formed at only one node; but if the stimulus is stronger, they form at additional nodes. The number of nodes on the main axis at which flower primordia appear is thus a

semi-quantitative measure of the effectiveness of treatment.

An example of an action curve is shown in Fig. 1. Five levels of energy were used in this experiment and the energies at each wave-length were plotted as indicated. Superimposed on these energy lines are the total numbers of flower primordia formed by lots of four plants which were irradiated with a specific wave-length band at different

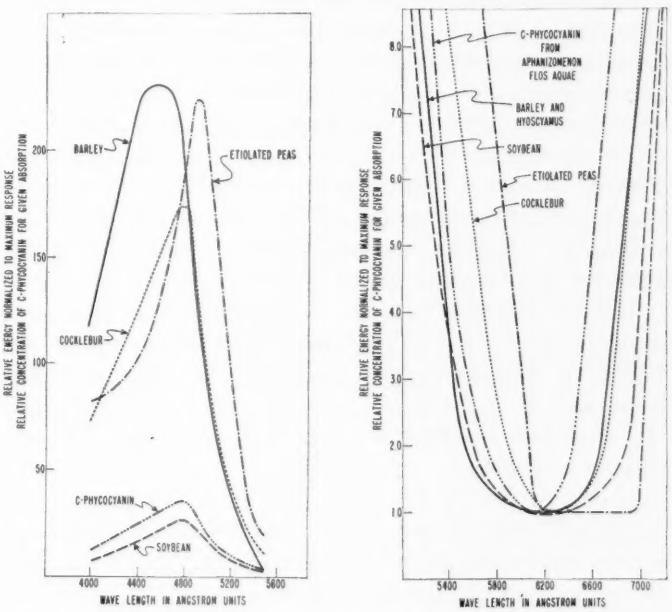


FIG. 2. Composite action curves for control of floral initiation of soybean, cocklebur, barley, and *Hyoscyamus*, and for regulation of leaf size in etiolated peas together with a curve showing the relative concentrations of c-phycocyanin for a given absorption.

energy levels. A response curve was drawn free-hand through the energy lines, crossing them between points at which sharp breaks occurred in the number of flower primordia formed.

All the action curves shown in Fig. 2 were derived in a similar manner. With cocklebur the criterion of effectiveness was whether or not the terminal bud formed flower

primordia (Parker, Hendricks, Borthwick and Scully, 1946). In barley the criteria were the stage of development of the spike and length of stem resulting from treatment (Borthwick, Hendricks and Parker, 1948). With *Hyoscyamus* the presence or absence of flower primordia and the length of stem that developed were used as measures of effectiveness (Parker, Hendricks and Borthwick, in press).

The action spectrum for the control of floral initiation in Biloxi soybean showed that all regions of the visible spectrum were effective if sufficient energy was provided. There were, however, two regions of maximum effectiveness: one in the red from 6,000 Å. to 6,800 Å. and the other, somewhat less effective, in the violet-blue region from 4,400 Å. to 4,000 Å. Minimum effectiveness occurred at 4,800 Å. This parallelled previous qualitative experiments with filtered radiation in various short-day plants (Kleshnin, 1943; Withrow and Withrow, 1940).

The action curve for control of floral initiation of another short-day plant, *Xanthium saccharatum* Wallr., is similar to that of Biloxi soybean, particularly for the regions of maximum and minimum effectiveness. Similar action spectra for the two short-day plants indicate that the same basic photoreactions are involved.

Action spectra for Wintex barley, *Hordeum vulgare*, and an annual form of *Hyoscyamus niger*, two typical long-day plants, were determined. The same technique of dark-period interruption, as used with the short-day plant, was employed. The action spectrum for barley (Fig. 2) was basically the same as that for the short-day plants. More energy was required, however, in the blue-violet region to initiate spike development than was required to suppress floral initiation in the short-day plants. The regions of maximum and minimum effectiveness, and the long wavelength cut-off coincided very closely. The response of *Hyoscyamus* in the red region of the spectrum coincided with that of other plants investigated. In the blue region the action curve was not definitely established; however, the position of minimum effectiveness seemed to be the same as that for the other plants studied.

The similarity of the action spectra for short- and long-day plants clearly demonstrates that the photoreceptor for the radiation must be the same pigment or a closely related one, as will be discussed later. While previous investigations (Katunskij, 1937) have postulated that these two classes of plants were operating through the same basic reaction the data on action curves clearly show their equivalence.

During the development of the technique for determining action spectra it was necessary to test whether or not response depended on the time over which the radiant energy was applied, because energy at any wave-band station was varied by varying time of exposure. While these experiments showed that the procedure used with the spectrograph was valid they also gave other useful information concerning the photoperiodic process. A small amount of energy applied over a 2-hour period had the same effect as more intense radiation applied for a few minutes, therefore, the dark-period recovery reaction following irradiation must be relatively slow. Additional information concerning the mechanism of the process was also derived from experiments in which a single brief interruption, with light of low intensity, was given to each of several lots of plants at intervals during the dark period. It was found that the energy required to prevent floral initiation in soybean and cocklebur was constant irrespective of whether irradiation took place one hour before the middle of the dark period or two hours beyond the middle. Interruptions during the first four hours of the dark period were ineffective because the dark period following the interruption was sufficient to permit initiation. Conversely, if the interruption to the dark period was delayed until 11.5 hours of darkness had elapsed some initiation occurred even at the highest energy level employed. These data, therefore, substantiate the observation that while the photoreaction preventing floral initiation requires only a brief exposure to visible radiation, the dark recovery is slow.

Threshold types of experiments were required to deter-

mine radiation levels for use with the spectrograph. In these experiments lots of plants were simultaneously exposed to different levels of visible radiation from any source. They give a quantitative approach to the effectiveness of such factors as temperature, gaseous environment, poisons, cycles of treatment, etc., to the photoreaction. Change of effectiveness can be measured as a shift in the threshold energy required to prevent floral initiation from that of the untreated plants. An example is lack of differential response of soybeans to absence of oxygen during dark-period interruption.

Non-Periodic Responses

Plants show many apparently non-periodic responses to visible radiation. There are indications that these might for the greater part belong to two principal classes; namely, those determined by radiation in the blue, such as phototropic response, and those having an action spectrum similar to that of floral initiation. An example of the latter is inhibition of second internode elongation and enhancement of leaf growth of dark-grown seedlings of pea, *Pisum sativum,* variety Little Marvel, by visible radiation. Control of elongation of the first internode of *Avena* appears to be similar in showing greatest effectiveness in the red portion of the spectrum and markedly less in the blue (Goodwin and Owens, 1948). In fact, this is the basis for suppressing elongation of *Avena* seedlings by use of red radiation during their growth for auxin assay.

Curtailment of internode elongation of seedlings emerging from the ground might in general parallel the behavior of pea and *Avena* seedlings. This response is not peculiar to seedlings of green plants for many albinos are known, by common experience, to show this type of response to visible radiation. The action spectra for several varieties of albino barley in this regard are now being determined, but none is known at the present time.

Chlorophyll formation, which probably involves transformation of protochlorophyll to chlorophyll (Smith, 1948),

in many plants is determined by radiation in the visible (Koshi, Smith and French, 1949). Response of protochlorophyll in dark-grown seedlings of *Zea mays* to radiation in the blue portion of the spectrum is approximately as great as that in the red for this reaction, which is in contrast to the curves shown in Fig. 2.

Growth of plants is known to depend strongly upon the ratio of intensities in the red and blue (Johnston, 1938), rather than solely upon the total radiation effective in photosynthesis. This has been one of the most difficult radiation responses to bring into quantitative expression. A specific example is afforded by soybean, where storage carbohydrate in the leaves is dependent upon the type of radiation (Parker and Borthwick, 1949) and can be changed at least two-fold by slight shifts in quality.

In each of the preceding examples enough is known about the light response to indicate its type. Several further phenomena are now mentioned for which this information is lacking but for which a sensitive response to prolonged darkness, or to illumination following darkness, is known. One of these is the predominant nocturnal growth in many plants, as, for example, tomato, under favorable temperature conditions (Went, 1944). Another is the enhanced growth of *Chlorella* in glucose media in light of very low intensity as contrasted to growth in darkness (Pearsall and Bengry, 1940), a more than two-fold difference being noted. Both of these responses resemble that of soybean, mentioned in the preceding paragraph, in involving carbohydrate utilization. An example of still a different type, is that of light influence on viscosity of protoplasm in *Elodea densa* (Stalfelt, 1946; Virgin, 1948), where incident energy as low as two foot-candle minutes is sufficient to produce saturation in a several-fold reduction of viscosity. The widely known and long appreciated conditions of shading for growth of some plants and need of full sunlight by others should also be held in mind and submitted to study.

The Pigment

The action spectra for all the biological responses under

discussion here are remarkably similar even though experimentally distinct in some details. They have the common characteristics of extending throughout the visible spectrum with a minimum energy requirement in the red and a maximum requirement, 25 to 250 times the minimum, near 4,800 Å. Response is negligible for wave-lengths beyond about 7,200 Å., which is about the limit of visible radiation. Resolving power was such that fine structure would have been detected within regions of less than 100 Å. in the red and 50 Å. in the blue portion of the spectrum. These features, interpreted through only the simplest photochemical considerations, indicate that the effective pigments either are identical or belong to the same class.

A pigment even without isolation can be characterized by its absorption spectrum, which gives the absorption coefficient at various wave-lengths. The action spectrum, being discussed here, which is expressed in terms of incident rather than absorbed radiant energy, will express the relative absorption spectrum of the pigment provided that:

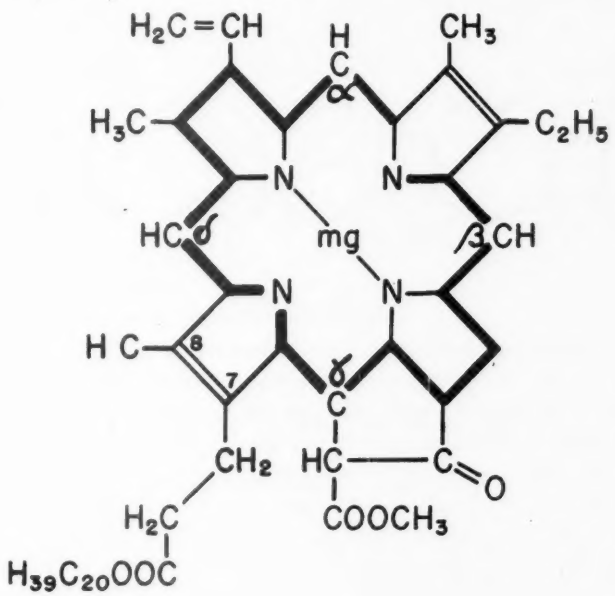
(1) The total light absorption is small, *i.e.*, absence of screening.
(2) Light quanta absorbed by the active pigment are equally effective independent of wave-length, *i.e.*, constant quantum efficiency.
(3) Light path in the plant is independent of wave-length.

The first condition, which refers to all pigments present, is not satisfied for any of the action spectra discussed in the preceding sections. It is most closely approached for response of dark-grown seedlings, but even then absorption due to carotinoids, in the blue portion of the spectrum, must be taken into account. Considerations about screening pigments, which are chiefly present in plastids, are not fully convincing under conditions where they account for most of the light absorption.

The paradoxical condition thus exists that even with the strikingly low effectiveness of radiation in the blue the absorption of the active pigment might be greater in the blue

than in the red or truly many-fold lower. This condition should be fully clarified by work now in progress with albino plants.

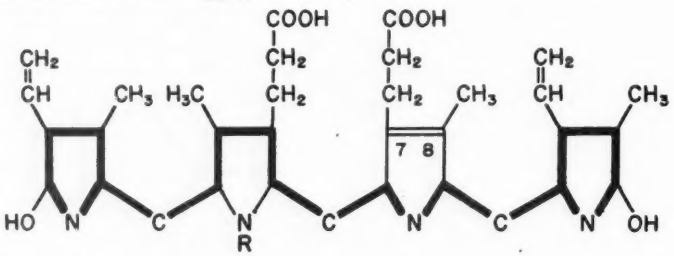
In view of the screening uncertainties at this time two types of pigment have to be considered. These are the cyclic and the open-chain tetrapyrroles. The cyclic tetrapyrroles, which are discussed first, include chlorophyll, protochlorophyll, and such ferroprotoporphyrin (heme) type pigments as cytochromes, catalase, and hemoglobins. These all have maximum absorptions in the blue, because of the so-called Soret band. Chlorophyll is essentially absent for some dark-grown plants, possibly including pea, and thus need not be considered further. The ferroprotoporphyrins that have so far been investigated all show



Protochlorophyll

several absorption maxima at wave-lengths longer than 5,000 Å., which are absent in the action spectra and which could not be removed by the possible screening of other pigments. The elimination thus leaves protochlorophyll or some unknown pigment of this general cyclic type, provided the absorption by other pigments is sufficient to reduce the incident light to about 0.002 of its initial intensity in the region of 4,500–4,800 Å. The formula of protochlorophyll is given at the bottom of page 129.

If the protoporphyrin ring is opened the absorption in the blue is markedly suppressed while that in the red is essentially unchanged. Ferroprotoporphyrin can be oxidized at the alpha position (Lemberg, 1935; Lemberg and Wyndham, 1936; Lemberg, Cortis-Jones and Norrie, 1938) to a bilin of the type,



Biliverdin

Proteins containing a bilin type of chromophoric grouping are the characteristic phycocyanin type pigments of the blue-green and the red algae (Lemberg, 1930). The related verdohaemochromogens, which give biliverdin upon acid treatments, have been observed as chloroglobins in animal erythrocytes (Foulkes and Lemberg, 1949; Lemberg, Lockwood and Legge, 1941) and are known to accompany catalase (Lemberg and Wyndham, 1936; Summer and Dounce, 1937). The chromophore pterobilin of this general type has been isolated from a protein separated from wings of the cabbage butterfly, *Pieris brassicae* (Wielland and Tartter, 1940). Oocyan, a blue pigment of some bird eggs, belongs

to this general class (Lemberg, 1931). Lemberg and his associates have been largely responsible for development of knowledge about the bilin protein types of pigments.

The relative light absorption of the Cyanophyceae-phycocyanin from *Aphanizomenon flos-aquae* (Svedberg and Katsurai, 1929) at various wave-lengths, normalized at the maximum of effectiveness in the red to the same relative scale as the action spectra, is shown in Fig. 2. This particular bilin protein was merely selected as an example. Agreement, without further considerations, with the action spectrum of soybean is almost exact, but is less so for etiolated peas.

If protochlorophyll is the effective pigment it is probably in the plastids and fully subject to screening by their pigments. Bilin proteins, on the other hand, if effective, would quite likely be in the cytoplasm and screening would be less important. In either case the concentration, indicated by isolation or attempted isolation, is very low and need not exceed the order of a few parts per million of the chromophoric group in the plant tissue and might be far lower. Considerable is known about the occurrence of photochlorophyll, while the bilin protein would quite reasonably have remained undetected because of low concentration and possible covalent C–N linkage of the chromophoric group to the protein (Lemberg, 1930).

Two possibilities exist for the role of the pigment in the light reaction, namely:

(1) It transfers the radiant energy to other reacting systems.
(2) It is a biocatalyst either before or after absorption of light.

The low concentration of the effective pigment and the very low incident energies required for biological control render unlikely the first possibility. In the second case, however, the action of the absorbed radiation could be greatly amplified. It is known that the concentration of protochlorophyll decreases upon illumination, possibly by

conversion to chlorophyll with addition of hydrogen atoms at positions seven and eight; but irrespective of the product the decrease is real. Protochlorophyll could thus very well fulfil the conditions of the biocatalyst. This possibility, however, exists equally for the bilin proteins where, too, addition of hydrogen at position seven and eight or in the vinyl group is reasonable. Here the biocatalyst might be either the bilin protein or its light product.

Photoperiodic Response in Animals

Photoperiod also influences the reproduction of some animals. The phenomenon is possibly of even wider occurrence in animals than plants, for it is known in birds, mammals, reptiles, fishes, amphibians, various invertebrates, and probably others (Rowan, 1938). The action primarily affects the reproductive structures and activities of the organisms. It also controls such characters as plumage type and color and time of molting of birds, or hair color and fur quality of mammals. The early literature on the subject has been summarized by Marshall (1936), Rowan (1938), and Bissonnette (1936).

The organ of photoperiodic perception in ducks (Benoit, 1934) and ferrets (Bissonnette, 1938) has been shown to be the eye. The stimulus is in some way transmitted from it to the pituitary, and through the latter the gonads are stimulated to activity. In the case of ferrets changes in hair cycles and sexual cycles are induced by appropriate light treatments. Both of these responses can be suppressed by removal of the pituitary. Removal of the gonads, however, inhibits the sexual cycles but not the hair cycles (Bissonnette, 1935).

Red radiation has been shown to be the most effective part of the visible for control of sexual cycles but quantitative relations have not been determined (Bissonnette, 1936). The effect has been attributed to the action of carotinoids in the eye, but in the absence of action spectra the nature of the pigment remains unknown. Action spectra for the response in animals are needed to clarify the

problem with respect to them. Such action spectra, moreover, will show whether or not the mechanisms responsible for photoperiodic phenomena in plants and animals are fundamentally alike.

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HISTOLOGICAL ASPECTS OF THE CHANGES FROM A VEGETATIVE TO A FLOWERING STATE[1,2]

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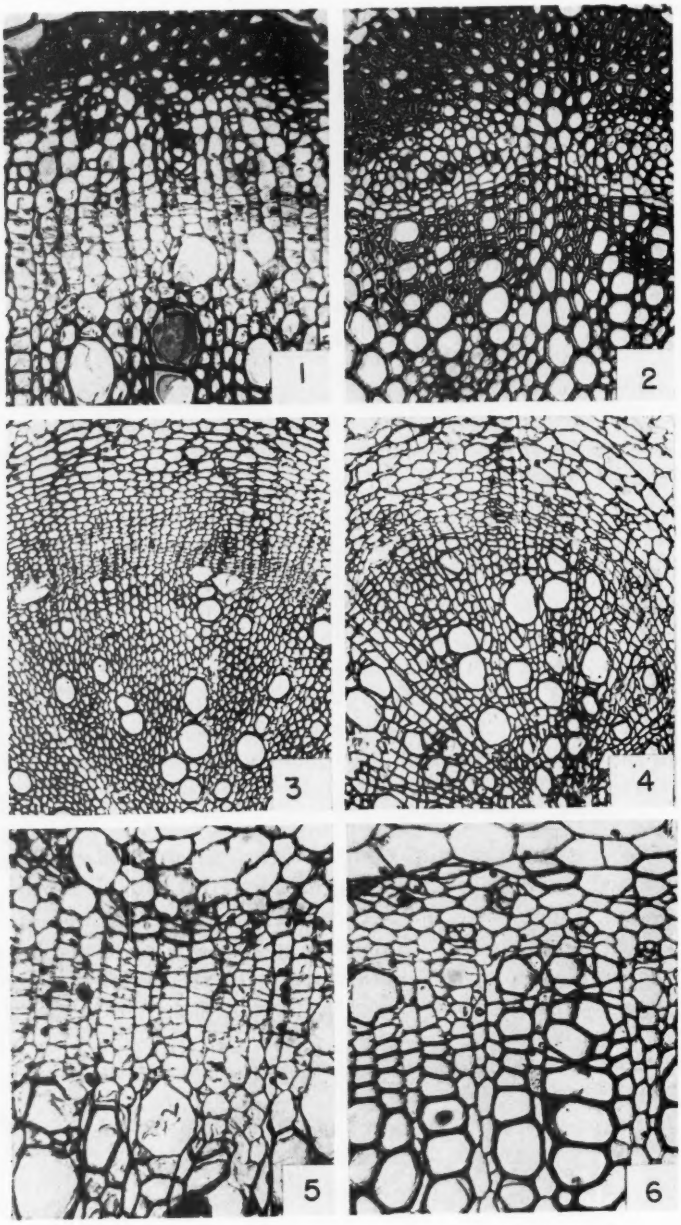
THE transition of plants from the non-flowering to the flowering state has been a subject of interest for many years. The problem has been studied from many angles. The one upon which we have centered our attention is the anatomical structure of vegetative and flowering plants.

The anatomical structure of plants in a vegetative and in a flowering state was examined and reported by Wilton and Roberts (1936). Although the plants required different environmental conditions to induce them to flower, the anatomical structure characteristic of the blossoming stage was similar in the species studied. In contrast to the non-flowering stem the flowering stem was characterized by: (1) a less active cambium; (2) thick-walled secondary xylem cells adjacent to the cambium in comparison to the thin-walled and differentiating xylem vessels in the non-flowering stem; and (3) cell walls generally thicker in the tissue of the pericycle, perimedullary zone and phloem (Figs. 1, 2).

The cambial and phloem tissue particularly underwent striking changes during the reproductive state. Wilton (1938) found that the cambium of vegetative plants remained active throughout the length of the stem. As the plants approached the reproductive stage the cambium ceased differentiation of phloem and xylem tissue, so that there were few meristematic cells in the stem. This study suggests the possibility that the annual habit in plants is

[1] Published with the approval of the Director of The Wisconsin Agricultural Experiment Station.

[2] Supported in part by a grant from the Wisconsin Alumni Research Foundation.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due to the cessation of cambial activity as the reproductive state is reached.

Since cambial activity in the stem of annual plants ceased at flowering (Struckmeyer and Roberts, 1939), we were interested in following the details of the changes in the phloem tissue. It was observed that: (1) in flowering plants some of the significant features of the phloem were the limited formation of sieve tubes and companion cells; (2) decrease in cell size and greater thickening of the cell walls; (3) crushing and obliteration of some of the primary phloem cells and the formation of phloem parenchyma rather than sieve tubes and companion cells.

Since the stems showed striking anatomical changes at flowering, preliminary examinations on roots of vegetative and flowering plants were made. Reduced cambial activity and generally thickened cell walls were characteristic of the roots from flowering annual plants (Figs. 3, 4). From these observations it was apparent that a conspicuous change in the anatomical structure of vegetative stems occurred.

Poinsettia blossomed in short days unless the temperature was 75° F. or above. However, if at 75° F. a current of cool air was passed through a chamber surrounding the third or fourth internodes, the plants flowered. The anatomical structure of the stem above and below the temperature chamber was examined. The internodes above the temperature chamber had acquired characteristics of a flowering stem. The cambium had become less active, and the thickening of cell walls was occurring. The internodes

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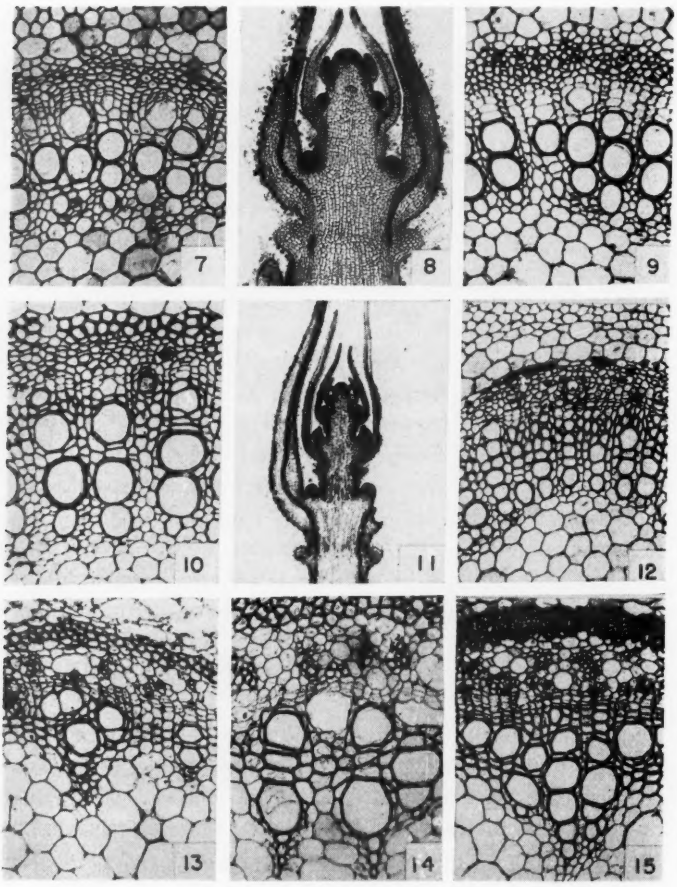
FIGS. 1–6. Transverse sections of stems and roots. FIG. 1, vascular bundle of stem of African Marigold. Active cambium giving rise to new vascular elements. FIG. 2, vascular bundle of flowering stem of African Marigold; absence of cambial activity; phloem cells smaller, and thickening of cell walls of vascular elements. FIG. 3, root from vegetative plant of tobacco var. Maryland Mammoth; active cambium apparent. FIG. 4, root from flowering plant of tobacco var. Maryland Mammoth showing decreased cambial activity. FIG. 5, fourth internode from vegetative plant of poinsettia; active cambium and differentiating elements apparent. FIG. 6, fourth internode of plant of poinsettia which was in a temperature chamber through which a current of cool air was passed; plants induced to flower; internode has less active cambium and a thickening of the cell walls.



FIGS. 7–15. Transverse sections of stems and longitudinal sections of stem-tips of *Salvia* and soybean. FIG. 7, fourth internode of vegetative plant with active cambium apparent. FIGS. 8, 9, stem-tip and fourth internode of *Salvia* given five short days; buds present in axils of leaves, whether they are blossom buds remains questionable; cells of cambial region less active and fewer vascular elements differentiating. FIGS. 10, 11, fourth internode and stem-tip of *Salvia* given eight short days; prominent blossom primordia and cambium less active. FIG. 12, fourth internode of flowering plant; cells smaller and thicker-walled, and little or no meristematic activity present. FIG. 13, fourth internode of vegetative plant of soybean; active cambium. FIG. 14, fourth internode of plant given five short days and returned to long days, five short days not sufficient to induce

from non-flowering plants showed cellular structures characteristic of vegetative stems.

The question then arose as to whether the anatomical changes were the result of flowering or if they appear with induction of blossom buds (Struckmeyer, 1941). Several species of plants were grown under environmental conditions that would induce flowering. Several short-day varieties as soybean, *Cosmos*, *Xanthium* and *Salvia* (of which the latter will be discussed) were studied. Samples of vegetative plants of comparable age were grown under long-day conditions to keep them in a non-flowering condition.

Plants of *Salvia* were grown in short days and the stem-tips and fourth internodes were sampled at regular intervals. Stems from vegetative plants showed structural characteristics of non-flowering plants (Fig. 7). The stems of plants given five short days showed less cambial activity resulting in a decrease in differentiation of vascular elements. The stem-tips of plants given five short days had inconspicuous blossom primordia (Figs. 8, 9). At eight short days prominent blossom primordia were apparent and the fourth internode showed a further reduction in cambial activity and vascular differentiation (Figs. 10, 11). After 39 short days the plants were in full flower; at this time the stem had smaller and thicker-walled cells; and cambial activity had ceased (Fig. 12).

Stock was induced to flower in long days when grown at a minimum temperature of approximately 65° F. The gradual change in the anatomical structure of the plant was apparent at about the same time blossom primordia were formed. From these investigations it is apparent that similar changes occur in the anatomical structure of the stem in a long-day plant during the transition from the vegetative to the flowering stage.

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plant to flower, and it retained vegetative characteristics. FIG. 15, fourth internode given twenty-four short days and fifteen long days, cells of stem have become mature. Twenty-four short days induced plant to extent that it did not revert to vegetative condition when returned to long days.

We were also interested in knowing what response was made by the stem-tips and vascular structure of plants that were exposed for a certain number of days to an environment favorable for flower induction and then returned to an environment favorable for vegetative growth.

Plants must be given a minimum length of the induction period to blossom; unless this is done the blossom buds will abort and the plants will again acquire vegetative characteristics. The number of days required for flowering varies with different species. Soybean var. Biloxi will be used as an illustration. Plants of soybean given five short days and then returned to long days showed no blossom primordia or decrease in cambial activity (Figs. 13, 14). Plants given 24 short days previous to their return to long days showed blossom buds that matured into flowers and fruit (Fig. 15). The structure of the stem likewise showed little meristematic tissue. From these observations it was evident that a sufficient length of induction must be given plants for blossom primordia to differentiate into flowers. It is not sufficient to secure only primordia, for unless the environment is favorable for their development, abortion will occur. With Biloxi soybean, 17 short days are needed to insure flowering.

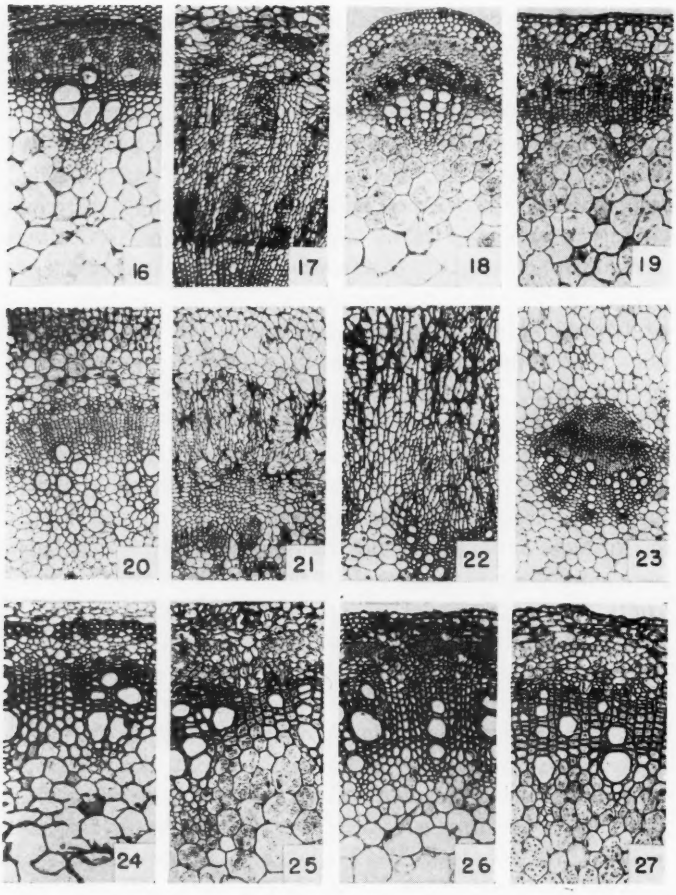
Species of plants flowering terminally were not induced to blossom by grafting the stem of a flowering plant onto a non-flowering one. On the other hand, species of plants which produced blossoms at the lower nodes also have been readily induced by the grafting technique.

Investigations on the effect of flowering on the boron requirements of plants that are photoperiod sensitive and those that are day-neutral were observed (MacVicar and Struckmeyer, 1946; Struckmeyer and MacVicar, 1948). Soybean var. Manchu was chosen because of its blossoming response when grown under short days. Plants grown with different photoperiods showed striking responses; those grown without boron and in long days showed a necrosis of the apical meristem and fragility of the leaves and petioles; those given a minus boron supply in short days fruited

and appeared essentially normal. The anatomical structure of these plants was examined. Plants grown in long days without boron showed an unusual arrangement of the cells due to abnormal cambial activity and proliferation of cells. The conspicuous difference was the increase in number and size of the phloem and ray cells. Considerable necrosis had occurred in these regions and the ray cells had stretched radially and increased in size. Xylem parenchyma was formed from the abnormal cambium before it ceased activity. Cells of the pericycle showed some tangential stretching, radial division and necrosis. Tangential divisions were apparent in the endodermis and cell enlargement in the cortex (Figs. 16, 17).

The anatomy of stems of plants grown with and without boron in short days was not greatly different. The characteristics of fruiting stems were apparent in plants grown in short days with boron; those grown without boron were also in the blossoming state and the effects of boron deficiency were only slightly apparent in the stems of these plants. There was no evidence of necrosis, the phloem was not greatly affected and the phloem parenchyma and ray cells showed some enlargement. The great increase in number of cells due to abnormal cambial activity and proliferation in plants grown in long days was markedly less in plants grown in short days and also without boron (Figs. 18, 19). These observations indicate that symptoms of boron deficiency were far more severe in long days than in short days where fruits were formed. The boron content of leaf tissue in boron-deficient plants in short and long days was very similar. In plants given one type of treatment the boron content appeared to be adequate while in a group given a different treatment severe boron deficiency symptoms occurred. The question then arises as to whether reduction in cambial activity associated with blossoming in short days could be responsible for the reduced need for boron.

Plants of tomato, a day-neutral species, grown in both short and long days, were in blossom 44 days after the start of the experiment, and showed the characteristics of flower-



FIGS. 16–27. Transverse sections of fourth internodes of stems of plants grown with and without a boron supply. FIG. 16, soybean (Manchu No. 3) grown in long photoperiod with adequate supply of boron. FIG. 17, soybean grown without boron in long photoperiod showing increased phloem, ray and xylem tissues from abnormal cambial activity and proliferation. FIG. 18, fruiting soybean grown in short photoperiod with boron; inactive cambium and thick-walled vascular tissue characteristic of fruiting stem. FIG. 19, soybean grown in short photoperiod without boron; slight increase in phloem and ray tissue. FIG. 20, blossoming tomato grown with boron in long photoperiod; anatomical structure normal for tomato stem. FIG. 21, tomato grown without boron in long photoperiod; abnormal proliferation of cells in cambial region and extensive necrosis. FIG. 22, cocklebur grown

ing stems. Another group of plants given the same treatment but grown without boron showed extreme cellular abnormalities. Abnormal cambial activity appeared to be the cause of this disorganization. There was enlargement and proliferation of cells in the phloem region, resulting in a disruption of tissue which formed the necrotic areas (Figs. 20, 21).

Soybean var. Pagoda is also an indeterminate variety in that it comes to fruit very early in both short and long days. It exhibited the anatomical features typical of fruiting stems under all treatments. There was no essential difference in the anatomical structure of plants given an adequate boron supply and those deprived of boron (Figs. 24, 25, 26, 27).

The boron requirement, when floral initiation was induced by exposure to only a few short days rather than continuous short photoperiods, was studied in cocklebur. Plants were given an induction period of four short days and then transferred to the long-day environment. Boron deficiency symptoms appeared shortly after the return of the induced boron-deficient plants to long photoperiods. Symptoms of boron deficiency were present both in those plants given an induction period of four short days and in those grown continually in long days in the absence of this element. The internal structure of stems of induced plants grown without boron showed severe disturbance in the cambial region. Some of the cells ceased differentiation and became enlarged; others continued to divide, but the derivatives did not differentiate into phloem elements but

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without boron, given four days of induction and then transferred to long days; cellular disorganization and necrosis apparent. FIG. 23, cocklebur grown without boron, given ten short days and returned to long days; no evidence of proliferation or other cellular disturbances. FIG. 24, soybean var. Pagoda grown in short days; almost complete cessation of cambial activity. FIG. 25, soybean grown without boron in short photoperiod; structure of stem normal with the indication of abnormal cambial activity. FIG. 26, soybean grown with boron in long days; anatomical structure typical of fruiting stem. FIG. 27, soybean grown without boron in long photoperiod; stems show fruiting anatomy and no cellular abnormalities.

remained meristematic. There was also evidence of necrosis. Another group of plants was given an induction period of 10 short days, after which they were transferred to long days. Those plants receiving 10 days of induction showed macroscopic flower buds 14 days after the beginning of the induction period. Three weeks later the minus-boron plants had aborting blossom buds and showed slight symptoms of deficiency. These symptoms were far less severe, however, than in those plants maintained in the vegetative state by continuous exposure to long days.

These observations revealed that internal abnormalities can be detected when external symptoms are only slightly evident or even lacking. In plants as cocklebur, buckwheat and soybean var. Pagoda, where cambial activity became inactive at an early stage when induced to flower, the symptoms of boron deficiency were repressed. In plants that come to flower slowly as tomato, the cambium remains active for a considerably longer time before flowering and the lack of boron thus has an opportunity to show its effects.

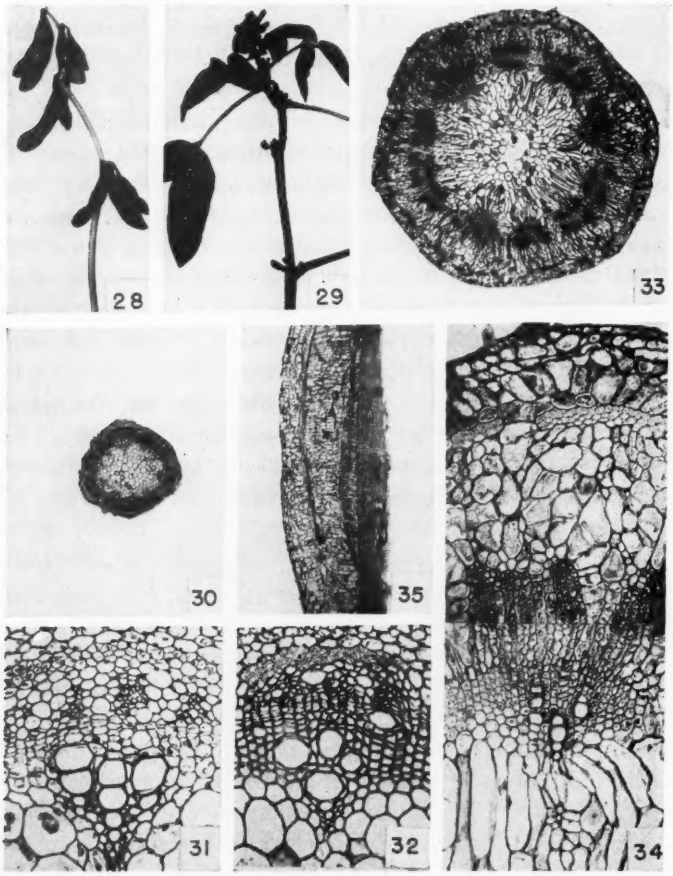
There have been many reports on the effect of 2,4-dichlorophenoxy acetic acid on the killing of weeds. The manner in which this chemical has its effect is through the increased proliferation of certain types of cells in the stems and leaves. Our experience with 2,4-dichlorophenoxy acetic acid has shown that this growth regulator has very little effect on flowering plants, while vegetative plants are severely stimulated. For example, in vegetative plants of *Xanthium* adventitious roots emerge through the cortex at both internodes and nodes. In flowering plants the tissues have matured to the stage where they are not stimulated to divide and proliferate as they do in the vegetative stem. In the nodal region there are a few meristematic cells which can be stimulated by this growth substance. The reason that many monocotyledons do not as a rule respond to 2,4-dichlorophenoxy acetic acid appears to be because of the lack of a cambial region. From our studies monocotyledonous plants that have a rudimentary cambium such as

Dracena, Anthericum and *Tradescantia* show the formation of adventitious roots when treated with 2,4-dichlorophenoxy acetic acid.

Another aspect of the relation of anatomy to flowering was the presence of abnormal swellings on the stems of some varieties of soybeans (Struckmeyer, 1946). Soybean var. Illini grown at the university greenhouses made an indeterminate response to photoperiod. When grown under short days and a continuously cool temperature (55° F.), or at higher day temperatures (65° F.) and cool nights (55° F.), swellings developed particularly on the third and fourth internodes of the stems (Figs. 28, 29).

The stems of normal fruiting plants had the characteristics of flowering stems as decreased cambial activity, decrease in the formation of phloem and xylem cells and a thickening of the cell walls of the vascular tissue (Figs. 30, 31, 32).

The diameter of the swollen stem of plants (called "dud" plants by growers) grown in short days and at a cool temperature were approximately three times that of the normal stem. Short, swollen and unripened pods at the nodes were also characteristic of these plants. However, when examining the cellular organization it was apparent that the increase in diameter was due primarily to the enlargement of cells and to a lesser extent the increase in number of parenchyma cells. The phloem parenchyma cells were greatly enlarged and frequently stretched in a radial direction. Earlier formed sieve-tubes and companion cells were crushed and obliterated by the enlarging parenchyma cells. The secondary sieve-tubes and companion cells were conspicuous in number and separated by vascular rays. There was a wide cambial zone, but some of the cells were not the strictly thin-walled tabular type which are characteristic of a normal cambial cell. There was a striking absence of vessels, most of the xylem elements being tracheids and xylem parenchyma. The medullary rays and pith cells were also large and misshapen. Endodermal cells enlarged tan-

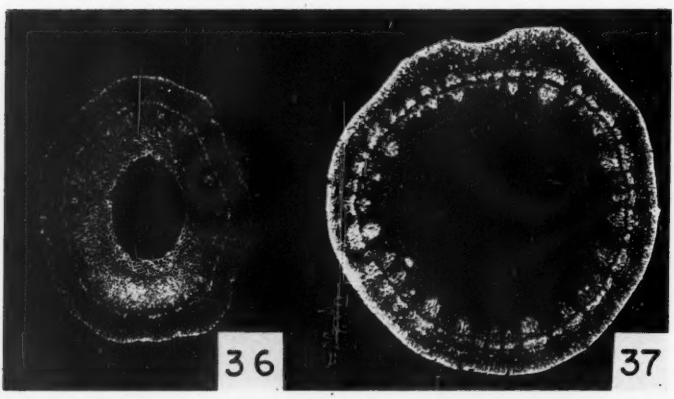


FIGS. 28–34. Normal and abnormal plants of soybean showing their fruiting habits. FIG. 28, normal plant of soybean (Manchu 606) showing natural leaf drop and mature fruits. FIG. 29, "Dud" plant of Manchu 606 of same age as plant in Fig. 28. FIG. 30, fourth internode of a plant grown in a warm temperature and in long days. FIG. 32, fourth internode of a flowering plant grown at a warm temperature and in short photoperiods. FIG. 33, cross-section of a stem of a plant grown at a cool temperature and in short days. Notice the difference in diameter and cellular structure of this stem compared with the normal (Fig. 30). FIG. 34, cross-section of a swollen internode; cortical cells have become somewhat stretched; conspicuous difference is the enlargement of the phloem parenchyma cells which lie below the phloem, and the wide cambial region. FIG. 35, longitudinal section and swollen region of internode; enlargement has occurred between the xylem and phloem fibers.

gentially between the vascular bundles and fiber groups, and divided above them (Figs. 33, 34). From longitudinal sections through the stem it was apparent that the radial swelling of the stems was due primarily to the enlargement of the pith and parenchyma cells and tangentially by stretching of the ray cells and enlargement and division of the endodermal cells (Fig. 35). These so-called "dud" plants were also found growing in the field when planting was early during cold weather. They showed a similar anatomical structure as those plants of soybean that produced swellings in the greenhouse. Frequently the epidermal and cortical cells became disrupted due to the enlargement of the internal tissues. An explanation of this type of plant behavior has not been made. The abnormal structure of the stem was not characteristic of flowering stems except under cool conditions. The increase in diameter of the stem was due primarily to increase in cell size. One might consider the possibility of the presence of a growth hormone, yet responses to growth hormones such as described by Kraus, Brown and Hamner (1936) were not characteristic of these stems even though they had the capacity to divide. For the present the cause of the swelling remains unknown. It apparently is not related to flowering, as plants grown in short days at a warm temperature, and plants grown in long days at different temperatures in which the stems remained normal were flowering.

Another test of the anatomical structure of the stem to flowering was determined when reversed night temperatures were used (Roberts and Struckmeyer, 1948). Several varieties showed this response and tobacco, var. Maryland Mammoth, will be used to illustrate the effects. It ordinarily flowers in short days. When grown in short days with a warm (65° F.) night temperature the plants come to flower. With the same temperature combinations, but in long days, the plants remained vegetative. The plants grown with reversed conditions of warm night temperatures and cool days soon became etiolated, but differentiated and developed blossoms at practically the same time as normal plants.

The mineral residue of plants following the microincineration treatment were studied. A greater concentration of mineral residue appeared to be present in flowering plants than in vegetative plants. This indicated that there was a redistribution of minerals or a change in mineral nutrition as the plants flowered (Figs. 36, 37).



FIGS. 36–37. Photomicrographs of incinerated sections of poinsettia. FIG. 36, mineral residue of vegetative plant. FIG. 37, mineral residue of flowering plant.

One result from the study of the anatomical conditions accompanying the reproductive state has been the development of a technique for the extraction of a crystalline substance from flowering plants, having physiological activity. This can not be obtained from non-flowering plants. This extract has been effective in inducing blossoming of *Xanthium* to about the same degree as one long photoperiod and it also inhibits the formation of callous tissue. The significance of this finding is: The natural hormone or hormone complex which induces blossoming causes maturation of tissue. This is in contrast to other hormones (*i.e.*, indoleacetic acid) which stimulate varying degrees of cell proliferation. The present extracts have not been fully characterized. Their identification should contribute much to an understanding of photoperiodism and its accompany-

ing conditions. Also, knowledge of these materials should aid in an understanding of the allied problem of the factors inducing blossoming of plants which are insensitive to photoperiodism.

In summary the following conclusions can be made: The anatomical changes of the stem associated with flowering are alike regardless of the environmental factors used to induce flowering. In flowering plants the cells of the vascular tissue have matured and become thick-walled. This may explain why some flowering plants are more resistant to treatment with growth regulators, for generally the cells stimulated with these chemicals have matured at the flowering stage. In some nutritional studies it might be important to work with vegetative plants, since the mature cells in flowering plants may not respond as readily to a minus mineral supply. From microincineration studies it is apparent that the mineral distribution is different in vegetative and flowering plants. We also know that the induction stimulus may be quite local, for when a temperature chamber with cool air is applied to one internode, the plant is induced to flower; and the anatomical structure above the temperature chamber is characteristic of flowering stems. Since it has been observed that anatomical changes occur or have occurred before blossom primordia are apparent, it tells us that when studying the physiology and chemistry of reproduction, one must begin as soon as plants are in an environment inducive to flowering.

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THE ROLE OF HORMONES IN FRUIT DEVELOPMENT

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IN order to make a discussion of fruit development complete one should discuss pollination, sterility, nutrition, chemical composition, respiration, the influence of various external factors, the role of hormones and numerous other aspects. This might require hours, and after questioning many persons and invariably getting different answers, I have decided to take advantage of the breadth of the topic as first suggested to me by Dr. Thimann and instead of attempting to discuss the whole topic of fruit development, confine myself to those aspects with which I am personally most familiar, namely, hormones.

As other speakers on this program will deal with the flower formation I can start with it already formed. Generally, pollination is the first step in the development of a fruit from the ovary of a flower. Seldom, if ever, is pollination alone sufficient stimulus, but if the pollen germinates and produces a pollen tube reaching some distance into the pistil, the chances of a fruit being formed are much greater. Hildebrand (1865), Müller-Thurgau (1898), Yasuda (1934), and others have reported that many times in cross-pollinations only a slight growth of the pollen tube is necessary for the formation of a fruit, which, however, will be seedless.

How is this to be explained in the light of the present-day hormone concept? In 1936 Gustafson (1936) was able to produce seedless fruits in *Lycopersicum, Petunia, Salpiglossis, Vallota, Begonia, Zephyranthes, Nicotiana, Agapanthes* and *Antirrhinum.* This was accomplished by applying such growth-promoting substances as indoleacetic acid, indolebutyric acid, indolepropionic acid and phenylacetic acid mixed with lanolin, to the style of the pistil.

These fruits were of approximate normal size, but they were seedless. The next year Gardner and Marth (1937) produced seedless fruits in the northern holly and the strawberry, and Hagemann (1937) was successful in doing the same with the gladiolus, and since that time many other investigators have repeated these experiments with other chemicals and other plants. Laibach (1932) and Thimann (1934) found that pollen contained growth hormones, and Fitting (1909), Yasuda (1934, 1935) and Gustafson (1937) demonstrated that extracts of pollen when applied to the pistil of some flowers caused enlargement of the ovary, and in some instances full-sized fruits were produced. Basing his arguments on the above information Gustafson (1939b) suggested that the initiation of growth in the ovary is caused by an increase in the growth hormone of the ovary as a result of pollination. According to this idea the pollen tubes transfer the hormone from the pollen into the ovary together with the sperm nuclei. Van Overbeek (1941) thought that the amount of growth hormone in the pollen would not be enough, and he suggested that the pollen supplied an enzyme which released hormones from the precursor. In this way a few molecules of an enzyme would release a large number of molecules of hormones. The hormone thus supplied to the ovary either directly or indirectly as a result of pollination acts as a trigger to set off growth reactions.

To gain further insight into the part played by pollination in the initiation of fruit development, Muir (1942, 1947) examined the hormone content of pollen, pollen tubes and pistils before and after fertilization. He found that pollen tubes contained many times as much hormone as the pollen, also that there is an increase in the hormone content of a pistil following pollination and that pollen contains an enzyme system which can release the hormone from its bound form in the tissue of the pistil. Thus the pollen tube brings into the pistil a large quantity of hormone and also an enzyme, which, acting upon the bound hormone in the cells of the pistil, releases still more hormone. The concen-

tration of hormone is now sufficient to initiate growth in the ovary and the development of the fruit has commenced. Within a few days after pollination there is evidence that the ovary has enlarged. Muir found that some of the hormone migrated toward the basal end of the pistil and he thinks some of it moves into the pedicel of the flower and there prevents the formation of the abscission layer. This initial growth set up in the ovary by pollination will usually cease unless fertilization also takes place.

That the retention of the flower on the plant is a hormone phenomenon has been indirectly shown by Laibach (1933), LaRue (1936), and Myers (1940), and directly by Gustafson (1938). The latter found that the injection of a hormone solution into the ovary of the Maryland Mammoth tobacco prevented the dropping of the flowers, even though no fruits were formed. Without pollination the flowers dropped within two days after opening. This has been observed in other plants. While many flowers retained on the plant do not produce fruits, yet their retention is necessary for the mobilization of food into the growing fruit.

While in general it is necessary for pollination and fertilization to be consummated there are a number of seedless fruits and many of them are produced without pollination. Webber (1929–30) states that fruits of the Washington Navel orange is produced without pollination, and the same is true of pineapple, banana, persimmon and many other fruits. However, most of our seedless fruits do require pollination and even fertilization may be required (Gustafson, 1942). If, as previously stated, pollination and pollen-tube growth are necessary for the initiation of fruit development, how is one to explain the situation in the orange, banana, pineapple and in others where there is no pollination? A study was made of the growth hormone content in the ovary of unopened flowers of a number of citrus fruits, and it was found that ovaries of those varieties of oranges and lemons which were seedless and did not need pollination had a considerably higher growth hormone con-

tent than those varieties in which pollination and fertilization were required (Gustafson, 1939a). It was found that the same was true in seeded and seedless grapes. It was therefore postulated that in those plants that do not require pollination for fruit development there is a concentration of growth hormone sufficient to set off the growth process without the augmentation of pollination.

The second important event in the fruit development is the fertilization. Murneek (1937) and others have observed that immediately following fertilization there is an increased activity not only in the flower but in the whole plant. Wittwer (1943) found that there was an increase in the hormone content in the kernel of corn following fertilization. Dorsey (1939) is of the opinion that double fertilization is necessary for complete development of the J. H. Hale peach. It is a common occurrence in this peach for "buttons," *i.e.*, small under-developed fruits, to be formed when blossoming is coincident with low temperature. Microscopic examinations of numerous "fruits" in early stages of development brought out the fact that when only the endosperm or the embryo developed in a fruit it was of the "button" type and only when both showed cell division and growth were the fruits of normal size for the stage of development. However, single fertilization did supply a stimulus for some growth. Tukey (1936) found that when he destroyed the embryo in late varieties of peach the development was interrupted. If the embryo was destroyed at an early stage of its development the fruit shriveled and dropped, but if the embryo was destroyed in a later stage the fruit remained on the tree and grew at a normal rate for some time, but stopped growing and ripened at a smaller size than fruits with seeds. The same author makes the statement that in the Downer cherry the largest embryos are found in the largest fruits (Tukey, 1933).

Horticulturists have found that early ripening varieties of cherries, peaches and plums do not as a rule have viable seeds. One can reverse this statement and say that varieties that do not produce viable seeds ripen early. Why?

Perhaps because due to the lack of seeds only a small amount of hormone is produced, which may reduce the mobilization of food into the developing fruit and set up ripening processes earlier than in varieties ripening seeds. It is well known that there is a correlation between seeds and size of fruit (Kraus, 1915).

From the above discussion it is obvious that embryos and seeds aid in the development of a fruit. Next let us look for an explanation. Dollfus (1936) found that ovules are very rich in growth hormones, and when he removed them from developing fruits there was nearly complete cessation of growth, but when lanolin containing indoleacetic acid was introduced into the ovary cavity the ovary continued to grow nearly as rapidly as when the ovules were present. Gustafson (1938) corroborated this with the crookneck summer squash. He found that when the part of the ovary in which the ovules are located was removed, there was very little growth, but if indolebutyric acid in lanolin was applied to the cut surface growth took place at a nearly normal rate, and the more ovules that were left the greater was the growth. Meyer (1936) and also Gustafson (1939b) have shown that ovules and seeds are very rich in growth hormones.

Loomis (1934) has found that ears of corn absorb carbohydrates from other parts of the plants to such an extent that there is a higher concentration in the ear than in other parts of the plant. Murneek (1939) has also found that developing embryos and fruits absorb food from the rest of the plant and may cause a decrease in the growth of the plant while the fruits are developing. Heinicke (1917) considers that seeds aid fruits in drawing food and water from the rest of the plant.

Swingle (1928) in explaining metaxenia considered that the embryo and endosperm produce hormones which are secreted into the ovary, and these hormones influence the metabolism of the developing fruit. Murneek (1939) considers that hormones may be responsible for the movement of foods from the plant body into the developing fruits.

Stuart (1938) found that bean cuttings dipped in indoleacetic acid showed a greater increase in total nitrogen in the basal portion and a greater decrease in the leaves than the control cuttings. He also found that the dry weight of the treated cuttings was greater in the basal region than that of the control cuttings. Mitchell and Stuart (1939), also using bean cuttings, found that the treated cuttings had a higher nitrogen content in the basal end than the untreated cuttings. Since all this nitrogen subsequently disappeared, these authors consider that the hormone treatment stimulated proteolytic enzyme activities.

All these experiments give quite conclusive evidence that growth hormones influence the translocation of foods. The developing seeds produce an abundance of hormone and thus stimulate the transport of foods into the ovary from the rest of the plant, as shown by a number of investigators. With this thought in mind it is easy to explain why there should be a relation between the number of seeds and the size of a fruit as stated by Kraus and why the peaches with incomplete fertilization or embryos killed would not grow to full size, but produce "buttons."

Munson, Ewert, Wolpert and others (Gustafson, 1942) have noted that in many species which naturally produce parthenocarpic fruits, these fruits are produced only when the plant is unusually vigorous or producing only very few fruits, so that the nutritive conditions are unusually good. Wolpert found that he was able to produce parthenocarpic fruits in *Solanum nigrum* only when he removed all but one flower per plant. It has also been observed that it is easier to produce parthenocarpic fruits in tomatoes by chemical treatment when the plant is bearing only a few fruits than when it is heavily loaded. To the writer it has always seemed that the food level has to be unusually high in order that fruits without seeds may be produced. We thus see that though in some plants and under some conditions fruits without seeds are produced yet in general seeds and their influence are necessary for the development of fruits.

That the prevention of the formation of the abscission

layer in the pedicel continues to be associated with hormones throughout the development has been shown by a number of investigators with peach, apple, plum and other fruits. (Gardner *et al.*, 1939.) This has been demonstrated by spraying the fruits several weeks before they were ripe. Sprayed fruits remained on the trees longer than unsprayed controls.

Several practical applications have been made of the knowledge we have on the hormone function in fruit development. It is a well-known fact that tomatoes grown in the greenhouse during the winter in the northern states do not produce a heavy crop. This is due to poor development of pollen, during the dark months of the year. Howlett (1941), Strong (1941), Murneek *et al.* (1944) and Roberts and Struckmeyer (1944) have developed practical methods for applying synthetic hormones to the tomato flowers. The hormones are usually applied as sprays, though other ways of application have also been used. Wittwer (1948) has found that 25 to 30 parts per million of *p*-chlorphenoxyacetic acid gives very good results. Indolebutyric acid, naphthaleneacetic acid and naphthoxyacetic acid have all been found to be quite satisfactory. In private communication Howlett has stated that the tomato growers have found that the hormone-produced fruits do not keep as well as seeded fruits and it may be that modifications will have to be made in the method of application or in the hormone used. Recently Blondeau and Crane (1948) and Stewart and Condit (1949) have reported that the Calimyrna fig, which usually requires pollination by the fig wasp, produced parthenocarpic fruits of normal color, size and taste when the syconium (cluster of flowers) was sprayed with 2,4-dichlorphenoxyacetic acid, 2,4,5-trichlorphenoxyacetic acid or indolebutyric acid. This procedure may prove to be very useful to the fig growers in California. If the chemical treatment proves to be satisfactory they can dispense with their unproductive pollen-bearing caprifig trees.

SUMMARY

One might summarize the previous discussion by stating that pollination and pollen germination result in an increased hormone concentration in the ovary, by the transfer of hormones and an enzyme which releases the hormone from its precursors, sufficient to start growth; and the subsequent fertilization and embryo development continues to produce hormones, which stimulate or perhaps direct the food translocation into the ovary, where the hormones also function in cell formation and cell enlargement.

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(Symposium continued in May–June issue)